Pro gradu -tutkielma
Maantiede
Luonnonmaantiede

VARIATION IN DIATOM SPECIES RICHNESS AND COMMUNITY COMPOSITION IN ROCK POOLS

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2018

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Matemaattis-luonnontieteellinen tiedekunta	Geotieteiden ja maantieteen laitos

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Työn nimi – Arbetets title) Title
Piilevien lajirunsauden ja yhteisökoostumuksen vaihtelu kalliolammikoissa

Oppiaine – Läroämne) Subject
Luonnonmaantiede

Työn laji – Arbetets art) Level	Aika – Datum – Month and Year	Sivumäärä – Sidoantal – Number of Pages
Pro gradu -tutkielma	Syyskuu 2018	93 s. + liitteet

Tiivistelmä – Referat) Abstract

Sekä ympäristö- että spatiaaliset tekijät säätelevät lajien levinneisyyttä pienen mittakaavan yhteisöissä. Mikroskooppisesta koostaan ja tehokkaasta passiivisesta dispersaalistaan huolimatta piileväyhteisöt ovat ympäristötekijöiden lisäksi vahvasti spatiaalisten tekijöiden säätelemiä. Ympäristö- ja spatiaalisten tekijöiden keskinäistä suhdetta paikallisille yhteisöille voidaan kuvataan metayhteisöteorian avulla. Tämän tutkimuksen tarkoituksena on selvittää ympäristö- ja spatiaalisten tekijöiden suhteellista merkitystä piilevien lajikoostumukselle ja yhteisöjen samankaltaisuudelle, sekä määrittää vallitseva metayhteisötyyppi rannikon kalliolammikkoyhteisöissä. Lisäksi selvitetään paikalliseen lajirunsausteen vaikuttavia tekijöitä. Kalliolammikot ovat graniittisia veden täyttämiä painanteita, joita esiintyy runsaasti rannikkokallioilla. Selvärajaisuutensa, hierarkkisen spatiaalisen rakenteensa ja vuorovaikutteisen lajistonsa ansiosta lammikot soveltuvat hyvin ekologisiin metayhteisötutkimuksiin.

Tässä tutkimuksessa tutkittiin 30 Itämeren pohjoisrannikolla sijaitsevaa murtovetistä kalliolammikkoa kolmen kuukauden ajan touko-kesäkuussa 2016. Tutkimusaineisto koostui benttisistä piilevänäytteistä, lammikoiden arvioiduista koordinaateista ja suhteellisesta eristyneisyydestä sekä veden fysikaalis-kemiallisista ominaisuuksista. Lajirunsauden ja yhteisökoostumuksen kannalta tärkeimmät ympäristö- ja spatiaaliset tekijät määritettiin yleistetyillä lineaarisilla malleilla ja redundanssianalyysillä. Ympäristö- ja spatiaalisten tekijöiden suhteellinen vaikutus yhteisökoostumukseen selvitettiin variaation osituksella. Lajiston samankaltaisuuden väheneminen etäisyyden kasvaessa määritettiin lisäksi Mantelin ja ositetun Mantelin testin avulla.

Kuukausittaisten GLM-mallien tulosten mukaan piilevien lajirunsautta selittivät parhaiten veden sähkönjohtokyky ja kalliolammikoiden sijainti. Etenkin lammikoiden etäisyys merestä vaikutti selvästi lammikoiden veden fysikaalis-kemiallisiin ominaisuuksiin ja lajirunsauteen. Variaation osituksen mukaan sekä ympäristö- että spatiaaliset muuttujat säätelivät merkitsevästi piilevien yhteisökoostumusta. Näiden muuttujien yhteisvaikutus yhteisöille oli toistuvasti suurempi kuin kummankaan muuttujaryhmän yksittäisvaikutus. RDA:n mukaan piilevien yhteisökoostumusta säätelivät pääasiassa veden sähkönjohtokyky ja lammikoiden etäisyys merestä, sekä vähemmässä määrin veden pH, kokonaisfosforipitoisuus ja lämpötila sekä lammikoiden eristyneisyys. Mantelin testien mukaan lajiston samankaltaisuus väheni merkitsevästi sekä lammikoiden välisen ympäristö- että spatiaalisen etäisyyden mukana. Ympäristötekijöiden vaikutus kasvoi kesän aikana, muodostaen suurimman osan yhteisökoostumuksen vaihtelusta ja lajiston samankaltaisuuden vähenemisestä kesä- ja heinäkuussa.

Tulosten mukaan kalliolammikoiden metayhteisöä voidaan parhaiten selittää massaefektillä. Tehokas passiivinen dispersaali tuo generalistilajeja paikallisiin piileväyhteisöihin, jotka vaihtelevien ympäristöolojen vuoksi koostuvat pääasiassa pitkälle erikoistuneista spesialisteista. Lajiston samankaltaisuuden väheneminen etäisyyden mukana sekä ympäristö- että spatiaalisten tekijöiden merkitys johtavat lajistoltaan heterogeenisiin ja lajirunsaisiin yhteisöihin, joissa spatiaalisten tekijöiden rooli on ympäristötekijöitä heikompi, joskin merkitsevä.

Avainsanat – Nyckelord) Keywords
metayhteisö, spatiaalinen, ympäristö-, murtovesi, mikrobi, dispersaali

Säilytyspaikka – Förvaringställe – Where deposited
Helsingin yliopisto, Kumpulan kampuskirjasto

Muita tietoja) Övriga uppgifter) Additional information

HELSINGIN YLIOPISTO – HELSINGFORS UNIVERSITET – UNIVERSITY OF HELSINKI

Tiedekunta/Osasto – Fakultet/Sektion) Faculty	Laitos – Institution) Department	
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Sonja Aarnio		
Työn nimi – Arbetets title) Title		
Variation in diatom species richness and community composition in rock pools		
Oppiaine – Läroämne) Subject		
Physical geography		
Työn laji – Arbetets art) Level	Aika – Datum – Month and Year	Sivumäärä – Sidoantal – Number of Pages
Master's thesis	September 2018	93 p. + appendices

Tiivistelmä – Referat) Abstract

Species are distributed to small-scale local communities by an interplay of environmental and spatial factors. As microbial species with efficient passive dispersal capacity and thus mainly under local environmental control, diatom communities nevertheless consistently show a strong spatial structuring as well. Different metacommunity perspectives have been developed to describe the relationship between environmental and spatial factors and their relative role for local communities. This study aims to examine the relative roles of environmental and spatial factors for diatom species composition and community similarity for defining the dominating metacommunity type among coastal rock pool communities. Factors affecting local species richness are also studied. Rock pools are granitic water-filled depressions occurring in high numbers on coastal rocky outcrops. Due to their clearly delineated borders, hierarchical spatial structure and interacting biota, rock pools are well suited for ecological metacommunity studies.

In this study, 30 brackish-watered rock pools located on the coast of the northern Baltic Sea were studied over a three-month period from May to July in the summer of 2016. The data consisted of benthic diatom samples, estimated pool spatial coordinates and relative isolation, and water physiochemical characteristics. Environmental and spatial factors most responsible for variation in species richness and community composition were determined with generalized linear models and redundancy analysis, respectively. The relative roles of environmental and spatial factors for community composition were quantified with variation partitioning. Distance decay in community similarity was further determined with Mantel and partial Mantel tests.

According to the results of monthly GLMs, diatom species richness was best explained by water conductivity and rock pool spatial location. Specifically, the distance from the sea had a clear role in regulating pool water physiochemistry and species richness. Variation partitioning revealed that diatom community composition was significantly explained by both environmental and spatial variables in each month. The joint effect of these factors was consistently larger than the pure effects of neither variable group. Environmental and spatial variables most in charge of diatom community composition in RDAs were water conductivity and pool distance from the sea, with a lesser impact of water pH, total phosphorus concentration, temperature and pool isolation. According to Mantel tests, community similarity was significantly related to both environmental and spatial distance between the pools. The influence of environmental factors increased during the summer, accounting for the largest share of the variation in community composition and distance decay of community similarity in June and July.

In the light of these findings, the significant role of both environmental ans spatial factors and the overall metacommunity organization in the studied rock pools are best explained by mass effect. It seems that while the variable physiochemical conditions maintain strong environmental control over the local diatom communities consisting of mainly highly specialized species, efficient passive dispersal acts as a continuous supply of more generalist species. This pattern, accompanied with significant distance decay of community similarity eventually leads to spatially heterogeneic and diverse communities with weaker yet significant spatial control.

Avainsanat – Nyckelord) Keywords

metacommunity, spatial, environmental, brackish, microbial, dispersal

Säilytyspaikka – Förvaringställe – Where deposited

University of Helsinki, Kumpula campus library

Muita tietoja) Övriga uppgifter) Additional information

Contents

1. INTRODUCTION

Microbial communities are responsible for a variety of biogeochemical processes vital for maintenance and functioning of biodiversity. Variations among these communities affect the whole ecosystem, and general knowledge of the mechanisms determining distribution and community composition of these microorganisms is urgently needed (Van der Gucht et al. 2007, Virta & Soininen 2017). Due to their high diversity, well-defined habitats, short generation times and geographically wide but locally clustered distribution, microbial algae have proved to be successful in testing different community-related ecological theories over short timescales (Smucker & Vis 2011). Study results of microbial algae allows scaling up several ecological phenomena to larger habitat types or organism groups (Stevenson 1996). So far, studies of microbial distribution and dispersal patterns in small-scale natural metacommunities still lack behind those of macro-organisms (Martiny et al. 2006, Vanschoenwinkel et al. 2008a, Meier & Soininen 2014). Geographical distance between microbial eukaryotic communities has rarely been assessed, highlighting the need of studies focusing on similarities between microbial communities (Verleyen et al. 2009, Östman et al. 2009). To date, few ecological studies on rock pool diatoms exist. Despite many descriptive marine studies from the outer archipelago of the northern Baltic Sea region, little is known of diatom community composition in small and eutrophic pools along a salinity gradient from weakly brackish to freshwater rock pools (Häggqvist & Lindholm 2015, Virta & Soininen 2017).

The hierarchical spatial structure of rock pools and their separate but interacting communities offer possibilities for metacommunity research (Srivastava et al. 2004, De Meester et al. 2005). Given the small size, shallow water, pristine nature and simple morphology with clearly delineated structure and occasional spatial connectivity, together with easily manageable biota with simple but representative food webs, these viable natural rock pool microcosms are easy to modify for scientific purposes (Blaustein & Schwartz 2001, Jocque et al. 2010, Boix et al. 2012). Global occurrence in large numbers and high densities along several geographical, environmental and ecological gradients makes rock pools highly available and statistically accurate model system for experimental studies and field surveys (De Meester et al. 2005, Brendonck et al. 2010). Representative sampling and quantification of community dynamics and dispersal processes is dramatically more effortless and more easily replicated than in larger and more complex aquatic systems and attained study results are comparable and easily interpolated to different spatial scales (Wilbur 1997, Srivastava et al. 2004, De Meester et al. 2005, Smith et al. 2005, Atalah & Crowe 2010, Spivak et al. 2011). Life cycles of pool species

are short, which facilitates the study of community dynamics often hard to detect among longer-lived species in other aquatic habitats (Brendonck et al. 2010). The potential of rock pools as ecological model systems has been proven in several studies during the last decade (Vanschoenwinkel et al. 2007). Relatively little is still known about the relative role of spatial and environmental processes affecting rock pool communities, and the ecology of rock pools remains poorly studied (Jocque et al. 2006, Verleyen et al. 2009, Firth et al. 2014).

Much of the recent debate in ecological literature has been attributed to the relative importance of environmental and spatial processes for metacommunities (Nabout et al. 2009). Recent ecological research has mainly been focused on local-scale factors affecting small-scale community structure and composition, ignoring any possible effects of regionality. Only recently has the focus shifted from strictly local control to cover species dispersal and other larger-scale processes. Studies combining testing of environmental and geographical distance and the relative roles of these two group of ecological factors for microbial species on a metacommunity context are rare (Martiny et al. 2006). Inclusion of spatial factors in ecological studies focusing on diatom community composition may thus improve the results by increasing the total variance explained by variation partitioning methods (Liu et al. 2016).

The aim of this study is to examine the patterns of diatom species richness and community composition in brackish-watered rock pools at the coast of the northern Baltic Sea. Specifically, the study aims to examine the relative roles of environmental and spatial factors for pool community composition and similarity. Based on the relative influence of these factors on the diatom communities, the dominating metacommunity types is determined for the studied rock pools. This study aims to find answers to the following research questions concerning rock pool diatom communities:

1) Which environmental and spatial variables affect diatom species richness and community composition in rock pools?
2) How do rock pool diatom community composition and similarity change along environmental and spatial gradients?
3) Which metacommunity perspective or a combination of perspectives best explain the observed variation among rock pool diatom communities?

2. SPATIAL ECOLOGY AND METACOMMUNITIES

A central focus of ecological research is the biogeography of organisms, best studied through interacting species populations and communities with various dispersal and extinction rates (Logue & Lindström 2008). Community ecology aims to explain species abundance, distribution and biotic interactions observed in nature through multiple environmental, spatial and temporal gradients (Leibold et al. 2004). At larger temporal and spatial scales, the concept of metacommunities comes into play, serving as a theoretical foundation for complex relationships between the abiotic environment and both local species richness – i.e. the number of species in a certain area – and community composition, the identity of current species in each locality (Heino et al. 2014). *A community* is defined as a set of interacting species communities occupying the same habitat patch, whilst *a metacommunity* consists of local communities occupying discrete habitat patches connected by potentially interacting species through dispersal. A patch, in turn, describes a finite area of a habitat occupied by local community. The size of a metacommunity is defined by the number of its interacting communities (Hanski & Gilpin 1991, Leibold et al. 2004).

Metacommunities are temporally, functionally and structurally dynamic, always involving some level of community-level interactions. A patchy distribution of communities with negligible among-patch dispersal does not yet constitute a metapopulation, as these communities are too isolated to form a unified metacommunity (Begon et al. 2006). In addition, metacommunities often exhibit a nested hierarchical spatial structure, with smaller communities being subsets of and enclosed by larger communities. Due to biotic interactions driven by different species-specific environmental preferences among and between communities, individual- and population-level changes in one community may alter these patterns in another community (Hanski & Gilpin 1991, Stevenson 1996). Habitat fragmentation is nowadays regarded as one of the leading forces behind formation of metacommunities: as natural environments are divided to discrete patches, local communities are geographically separated, yet some interaction may be enabled via a few connections between these formerly continuous habitats (Hanski & Gilpin 1991). These remaining connections are responsible of maintaining species dispersal patterns over the fragmented landscape, eventually creating a single metacommunity of assemblages otherwise geographically isolated from each other.

2.1. Species dispersal and distribution

Species dispersal refers to spatial spreading of species from one place – bet that a habitat, a community or an ecosystem – to another, referred to as emigration and immigration, respectively (Leibold et al. 2004). Dispersal usually results in widening of species distribution range. Species are divided into two categories by the dominant means of their movement and dispersal. Active dispersers are a group of species capable of traveling from patch to patch on their own and immigrating themselves to new habitats. These species usually have wings or other structural adaptations to allow effortless dispersal. Since active dispersers are free to escape from unsuitable habitats or unfavorable environmental conditions, they usually have high colonization rates and wide distribution ranges. Passive dispersers, instead, are a group of species uncapable of moving and dispersing themselves to new habitats by their own means. These species lack wings or other morphological structures enabling movement, relying completely on other species or natural forces for dispersal. While active dispersers have the capability of choosing their target site, passive dispersers must thrive at the microhabitat they happen to land on by chance, complicating successive establishment of a new community (Shurin 2000).

The dispersal capacity of species is also determined by the distance the species is capable of traveling either passively or actively. Long-distance dispersal is especially typical of active dispersers, leading to more homogenic communities by species composition compared to those of passive dispersers (Soininen 2016). Nowadays it is assumed that the dispersal capacity of passively dispersing species is size-dependent, with smaller and lighter species and individuals more easily carried by dispersal vectors such as wind, water and animals (Vanschoenwinkel et al. 2008a, Naselli-Flores et al. 2015). Dispersal barriers further prevent species from dispersing to an area. Spatial barriers refer to geographical distance between sites, defining the degree of isolation of a given habitat. Environmental barriers, on the other hand, refer to abiotic and biotic differences in environmental conditions between sites such as alterations in climatic conditions or in species interactions. The relative efficiency of a barrier in preventing dispersal is often species-specific. For example, a rock pool with discrete vertical boundaries may serve as an effective physical dispersal barrier for aquatic planktic species, while larger organisms capable of flying are free to emigrate from the pool (Leibold et al. 2004).

Several factors regulate species presence and distribution patterns in a habitat, interacting and overlapping with each other through several spatial and temporal scales. Former theories

concerning species distribution patterns were strict in their assumptions of species being distributed to their habitats solely by local environmental processes. Nowadays the theory holds that species distributions are determined by both local environmental and regional-scale spatial factors, with species-specific variations in the relative importance of these two interacting components in distributional patterns (Ricklefs 1987, Poff 1997, Hillebrand & Blenckner 2002). The effect of dispersal-related processes on a community largely depend on the spatial scale, while local abiotic and biotic factors may also largely fluctuate on a temporal gradient (Begon et al. 2006, Heino et al. 2014). The influence of spatial control decreases with scale, giving room for environmental factors mostly responsible of community control on small-scale habitat patches (Green et al. 2004, Martiny et al. 2006, Soininen 2007, Soininen et al. 2007a, 2011, Bottin et al. 2014). Over long time periods, all species are likely able to disperse to a local community due to sufficient amount of time for needed ecological adaptation to evolve within species in the global pool. As the time available for colonization of a local community decreases, smaller share of species in the global pool are capable of joining the local community due to limited time for development of species evolutionary adaptations for prevailing small-scale ecological conditions. Thus, understanding of ecological phenomena behind a community requires considering multiple temporal and spatial scales relevant to a given community (Poff 1997, Logue et al. 2011, Heino et al. 2014).

In determining species distributions, understanding the extent to which communities are regulated by either environmental or spatial factors is the key for quantifying the metacommunity dynamics of different organism assemblages (Liu et al. 2016). In short, to reach and colonize a local community, suitable sites need to be available for the species to establish itself, and the species must be capable of dispersing to the site with a probability of immigration exceeding the probability of being restricted by dispersal barriers (Shurin 2000). Once arrived at the site, abiotic resources must be of sufficient supply and biotic interactions non-exclusive for a successful colonization to take place (Stevenson 1996, Begon et al. 2006). If these requirements are not met, the probability of the species to extend its distribution range will be low. The spatial and temporal interplay between scaled habitat features determining species distributions is often described as a combination of three nested but partly overlapping ecological filters. These are scaled habitat features, acting at different spatial and temporal scales and selecting species with appropriate traits from a global species pool – i.e. the sum of all species at a global scale – to specific habitats to form a local community (Poff 1997, Zobel 1997, Lawton 1999) (Fig. 1). To enter a local community, species must first pass through the

largest-scale *history filter* consisting of global climatic, geological and evolutionary factors such as speciation, extinction and migration (Cornell & Lawton 1992). Passing through the first filter sets species under regional-scale spatial control of dispersal distance and -ability, comprising the second, *dispersal filter* (Hillebrand & Blenckner 2002, Soininen et al. 2007a). Regional species pool has a spatial extent far larger than that of a local community; hence, not all species in the regional pool fulfilling the requirements of the dispersal filter are able to survive in large numbers in the local community (Poff 1997, Lawton 1999). Thus, at the finest spatial scale, an *environmental filter* consisting of local abiotic habitat characteristics such as physical and chemical conditions and their fluctuations, environmental disturbance and biotic factors such as predation and competition act as the final selecting force for species composition of the local community (Cornell & Lawton 1992, Hillebrand & Blenckner 2002, Soininen 2007). The species capable of passing through all the three filters are the ones that are best adapted to local conditions and have the highest probability of surviving in the local community, comprising the small local proportion of the global species pool (Poff 1997).

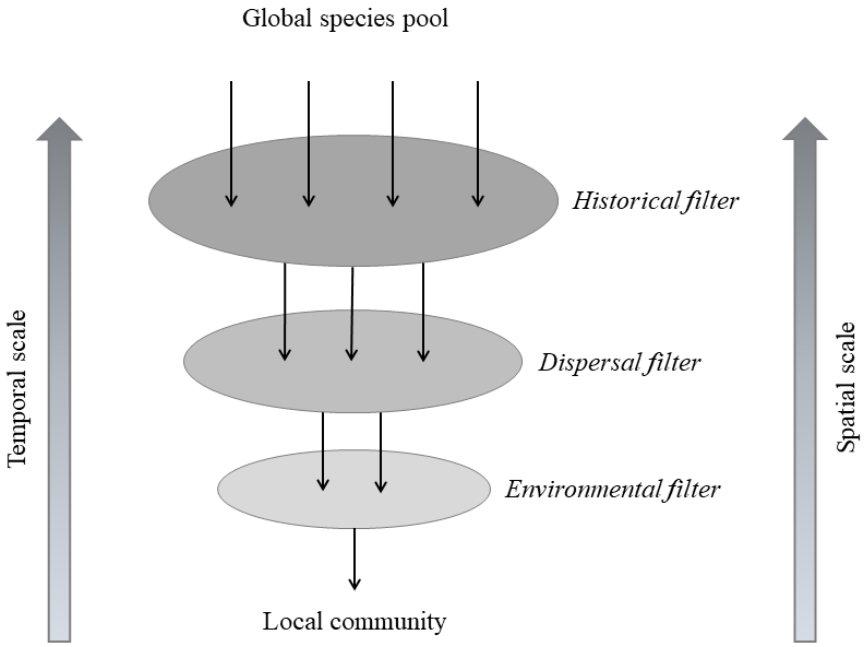


Figure 1. Schematic picture of the three hierarchical ecological filters (horizontal ellipses) selecting species to a local community from a global species pool. At the largest spatial scale, species must first pass through a *history filter*, determining species with potential to colonize available habitats through speciation and extinction events. The second, regional-scale *dispersal filter*, is passed only by species with sufficiently good dispersal abilities to enter suitable habitats. At the smallest, local scale, species must yet to correspond environmental requirements of their habitat set by the *environmental filter* in order to survive and reproduce in a local community. The temporal scale decreases when passing through the nested filters. The black vertical arrows describe the relative number of species capable of passing through the filters, decreasing along the spatial scale. The decreasing length of the arrows along spatial scale reflects the decrease in species abundance. Modified after Poff (1997) and Soininen et al. (2007).

2.2. Distance decay of community similarity

The first law of geography introduced by Tobler (1970) assumes that similarity of observations is negatively related to the distance between them. This assumption lays base to the theoretical concept of distance decay of community similarity, introduced by Nekola & White (1999). Distance decay describes the decrease of biodiversity along an increase in both spatial and environmental gradient typical of several ecological phenomena. The similarity of species between sites decreases both monotonically and linearly with increasing distance due to species-specific variation in dispersal ability and ecological niche, respectively, becoming more evident when moving farther apart along the spatial or environmental gradient (Nekola & White 1999, Condit et al. 2002, Soininen et al. 2007a).

Studying patterns of beta diversity, the variation of species composition between communities (Baselga 2010), offers possibilities for determining the relative importance of environmental heterogeneity, dispersal limitation and historical events in structuring communities (Green et al. 2004). *Beta diversity* (Whittaker 1960) is quantified by dividing gamma diversity – i.e. the total species richness of a region – by mean alpha diversity – i.e. the number of species in a certain site. Beta diversity is divided to species turnover and nestedness, the former describing replacement of some of the species present in one site by other species on another site (Baselga 2010). Beta diversity is influenced by many factors from ecosystem productivity, environmental heterogeneity, climatic patterns and evolutionary time to fluctuations in abiotic conditions (Begon et al. 2006).

Beta diversity can be quantified as distance decay in community composition (Nekola & White 1999, Soininen et al. 2007a, Wetzel et al. 2012). Differences in community similarity arise from gradual change in spatial or environmental barriers between habitat patches in a metacommunity (Jenkins & Buikema 1998). The spatial gradient simply refers to geographical distance between habitats, while the environmental gradient describes gradually changing environmental conditions with respect to both abiotic and biotic factors. Ecological requirements and tolerances of a species, referred to as ecological niche, vary according to species-specific tolerances and adaptations to different habitats (Begon et al. 2006). Sites closer to each other on the gradient share more similar environmental conditions than sites farther apart, resulting in local biota with more similar ecological requirements (Nekola & White 1999). As the distance between habitats increases, so does environmental variability, leading to changes in species composition due to differential ecological requirements. Likewise,

geographically close sites often lack major barriers for dispersal, resulting in more similar biota between habitats. Moving farther along the distance gradient introduces growing number of dispersal barriers, restricting species distributions into spatially delimited, heterogeneous habitats with the number of shared species decreasing with distance (Nekola & White 1999). Limited dispersal or high environmental heterogeneity results in faster decrease in community similarity along both distance gradients, serving as an implication of more pronounced turnover and thus higher beta diversity between sites (Chave 2004, Green et al. 2004, Soininen et al. 2007a).

Specific methods have been developed to describe patterns of distance decay between communities. *Halving distance* describes the exact geographical distance at which half of the species has been replaced by other species (Soininen et al. 2007a). In other words, the similarity of species composition has decreased by 50% from the original maximum, initial similarity. *Initial similarity*, in turn, describes beta diversity, or species turnover as such (Soininen et al. 2007a). Halving distance and initial similarity give cues about distance decay and species turnover more effectively than traditional correlation coefficients. These two concepts effectively describe the rate of change in species composition in relation to geographical distance between communities. A community whose species composition changes rapidly along the distance gradient has low initial similarity, short halving distance, high turnover rate and consequently high beta diversity, seen as a steep curve on the plot describing the relationship between distance and species composition (Fig 2). Conversely, a community whose species composition experiences only minor changes with decreasing distance, has more gently sloping curve, higher initial similarity, longer halving distance and a lower turnover rate and beta diversity (Wetzel et al. 2012).

The observed strength of distance decay partly depends on the spatial scale: considering only part of the environmental or spatial gradient may lead to underestimation of the decrease in community similarity, as the small-scale heterogeneity may create discontinuities along the gradient which would at larger scale result in a significant distance decay pattern (Nekola & White 1999). Small study extent, highly variable environment, high degree of habitat isolation, large number of dispersal barriers and low dispersal capacity occasionally accompanied with small body size and consequently shorter life cycles and more rapid responses to changing environment will all result in spatially heterogenic species assemblages, rapid turnover and thus strong distance decay in community similarity (Soininen et al. 2007a, Wetzel et al. 2012).

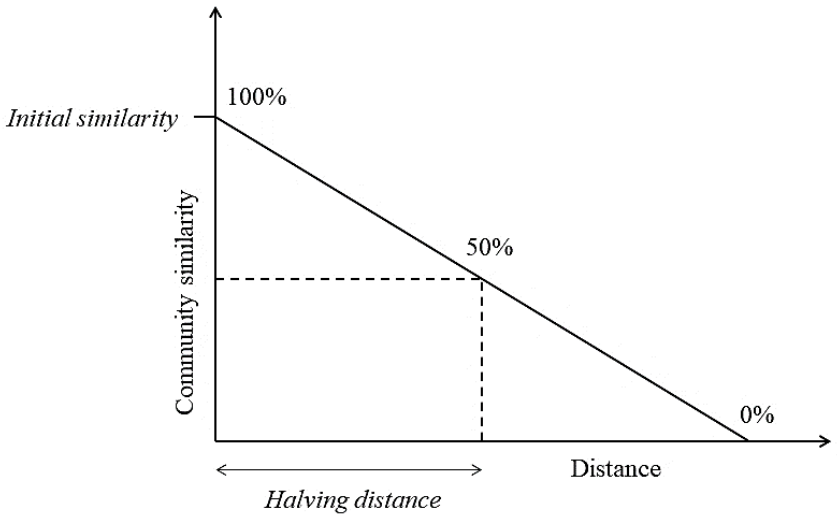


Figure 2. Conceptual diagram of distance decay of community similarity. Community similarity may decrease both along environmental and geographical distance. In the case of the latter, initial similarity reflects the community composition at 1-unit distance. Halving distance is the geographical distance at which half of the species in the original community have been replaced by other species. At that distance, 50% of the initial similarity has been lost in the community. As the geographical distance increases, communities become more dissimilar in their species composition, eventually having no species (i.e. 0%) in common. Modified after Soininen et al. (2007a).

2.3. Metacommunity theory

A good ecological theory is one that can be conveniently applied to different communities and organisms over multiple spatial scales (McGormick 1996). Through the application of metacommunity theory, community-level patterns in species richness, abundance and distribution as well as in biotic interactions are tightly coupled and explained against theoretical background of spatial and environmental processes on a scale of metacommunities (Leibold et al. 2004, Logue & Lindström 2008). Current metacommunity theory is divided to four partly contrasting conceptual paradigms, each reflecting variable relative roles of environmental and spatial factors in regulation of metacommunity dynamics and species composition in natural communities (Logue et al. 2011) (Fig. 3). Of these four perspectives, three are applied to explain patterns of variation in the rock pool diatom communities of this study. The fourth perspective, that of *patch dynamics,* falls outside the theoretical framework of this study and is thus not considered in more detail in this thesis.

Neutral theory, first introduced by Hubbell (2001), stresses the importance of spatial processes in regulating metacommunities and is concerned of demographic stochastic events affecting communities (Chave 2004, Cottenie 2005, Logue et al. 2011, Rosindell et al. 2012). As the name implies, neutral theory has its foundation in a view of species being equal in their

environmental constrains. Species are distributed to their environments by dispersal alone, driven by global speciation and extinction and their local-scale counterparts, emigration and immigration (Logue & Lindström 2008). Thus, all species with similar environmental tolerances have equal possibilities to disperse in, colonize and reproduce in any possible habitat, with no distinct, strictly delineated ecological niches determining species adaptation to different environmental conditions (Chave 2004). Random stochastic events are the only determinants of species distribution, and in the lack of niche differentiation, all species may be found from every possible patch in a habitat. Besides individual species being equal in their ecological requirements, habitat patches are likewise similar with little variation in their environmental setting (Leibold et al. 2004). Thus, local communities are rather similar in species composition (Vanschoenwinkel et al. 2007, Heino et al. 2010). Neutral theory is often seen as a basic theoretical framework whose existence among communities can be complemented by the three other metacommunity perspectives (Chave 2004).

According to *species sorting*, local abiotic environmental factors are responsible of the occurrence and distribution of species to local communities. Each species has their own tolerances toward environmental conditions expressed by ecological niches, separating species to environmentally varying habitat patches. In addition to these species-specific adaptations, habitat patches differ markedly in their abiotic characteristics, forming highly heterogenic environment (Leibold et al. 2004, Logue & Lindström 2008). Unlike under neutrality, species are capable of reaching all possible habitat patches. Although constant, however, species dispersal is not high enough to hold the dominating role in community regulation and affect species distribution patterns (Logue et al. 2011). Instead, environmental factors are the most responsible for controlling community composition and species diversities, causing clear distinctions between local communities (Heino et al. 2010). Although involved in community structuring, dispersal acts in the shadow of environmental processes, enabling species to reach suitable sites while not resulting in independent spatial patterns in a metacommunity (Cottenie & De Meester 2004, Cottenie 2005, Vanschoenwinkel et al. 2010, Heino et al. 2014).

The *mass effect* perspective (Shmida & Wilson 1985) involves both species dispersal and environmental factors as being responsible for species community composition. As in species sorting, species and habitats differ in their characteristics, being far from homogenic in relation to environmental conditions (Leibold et al. 2004). As environmental preferences expressed by ecological niches divide species to their local habitats, dispersal between patches maintains the viability of communities by enabling temporal species occurrence outside their natural

distribution ranges (Pulliam 2000, Logue et al. 2011). Increasing rate of dispersal not limited by natural barriers acts as an efficient supply for individuals from a natural species reserve, occasionally widening species distribution ranges over the limits set by environmental tolerances and adaptations (Leibold et al. 2004, Cottenie 2005). This ensures species survival in habitats environmentally less suitable for their biota and rescues species from competitive exclusions in communities with many ecologically similar species, simultaneously allowing species to exist in communities despite their negative growth rate (Pulliam 2000, Cottenie 2005, Winegardner et al. 2012). The resulting metacommunity consists of communities with partly homogenic species compositions, regulated by both environmental and spatial gradients (Logue & Lindström 2008, Heino et al. 2014). Thus, local communities are a mixture of both species with adaptations restricting them to these exact habitats, and species occurring in the habitat solely due to excessive dispersal maintaining high abundance in the community despite the lack of suitable adaptations.

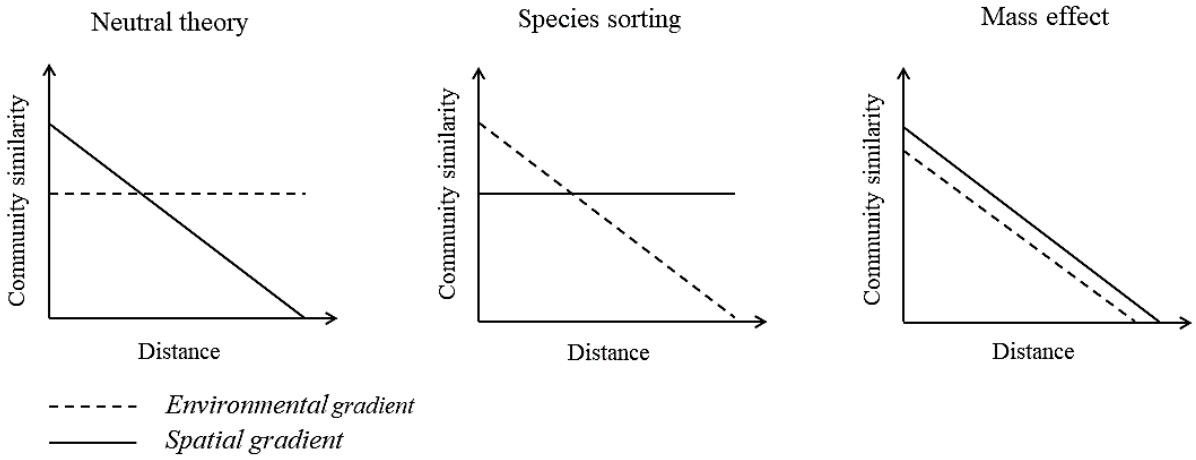


Figure 3. Conceptual diagrams describing the relationship between community similarity and environmental and geographical distance according to three metacommunity perspectives. In communities under *neutrality*, community similarity decreases along geographical distance due to spatial limitation of species, while environment has no effect on species with equal niches. In *species sorting*, community is affected by both spatial and environmental processes, but the role of environment is more pronounced due to ecological differences among species, and community similarity mainly decreases along environmental distance. Under the control of *mass effect*, dispersal and niche differentiation are both high, and community similarity decreases both with environmental and spatial distance. Due to effective dispersal, species may temporarily habit less suitable sites or escape competitive exclusion to sites allowing coexistence with ecologically differing species. Modified after Logue & Lindström (2008).

In real world – and especially at the scale of communities – each ecological theory is true only under particular circumstances, and no theory is scale-independent (Levin 1992, Lawton 1999). The real drawback of any ecological theory is that similar patterns can be – and often are –

generated practically by many other theoretical models reflecting natural processes (Rosindell et al. 2012). Especially at community level, determining a single universal process creating the observed patterns in nature is practically impossible. Thus, none of the presented metacommunity perspectives can probably explain all observed patterns in nature and hence are not truly conflicting with each other (Levin 1992, Lawton 1999, Chave 2004). Natural metacommunities are nearly unexceptionally constrained by a combination of both environmental and dispersal-related processes, with gradual changes from one metacommunity type to another. Any combination of the four existing paradigms may be responsible for observed metacommunity dynamics, determining the relative role of environment and space for a metacommunity (Leibold et al. 2004, Cottenie 2005, Logue et al. 2011). According to current opinion, the whole concept of metacommunity theory is best described by neutral theory and species sorting, the two other perspectives – those of mass effects and patch dynamics – being only special cases of species sorting (Winegardner et al. 2012). The fundamental difference between neutrality and species sorting is that while the former assumes species to be equal in their traits, the latter requires species to differ from each other either by their ecological tolerances (Leibold et al. 2004). The role of geographical distance in limiting species existence in a community forms a U-shaped relationship with species dispersal rate, decreasing in importance from communities under neutrality toward species sorting, and increasing again from species sorting to mass effect (Verleyen et al. 2009, Winegardner et al. 2012).

The relative dominance of the metacommunity paradigms in community assembly is affected by different factors ranging from spatial scale and geographical distance between different habitat types to species-specific dispersal ability and morphological characteristics (Cottenie 2005, Heino et al. 2014). This relative role of the three paradigms can be quantified through statistical methods separating the pure and joint effects of environmental and spatial variables on species community composition. According to Cottenie (2005), the significance of these two components for community composition determines the dominating metacommunity perspective for a set of communities. The method involves partialling out the pure and shared effects of environmental and spatial effects – i.e. ecological niche and dispersal-limitation, respectively – for local species communities and separating between statistically significant and non-significant effects of these variables for community composition. If only the pure effects of spatial factors independent of environmental variables are significant, communities can be regarded as spatially restricted, with dispersal limitation being most responsible for species diversities and distribution. This would support the theory of neutrality, with non-existent

effects of environmental constraints on local communities. If only the pure effects of environmental factors independent of spatial ones are to be significant, communities are mostly regulated by local habitat differences unaffected by spatial distances among sites. If both the pure environmental and pure spatial components show signs of significance for a species community, mass effect is the likely source of variation in community composition, resulting in local communities being affected by the joint effect of environmental habitat heterogeneity and dispersal limitation.

3. DIATOMS

Diatoms (Bacillariophyceae) are unicellular eukaryotic algal organisms belonging to the superphylum of Heterokonta (Heino et al. 2010). Diatoms are an exceptionally diverse and most species rich group of microbial eukaryotic algae, with a global species richness estimated as approximately 200,000 species belonging to over 900 diatom genera (Mann & Droop 1996, Vanormelingen et al. 2008b). In freshwaters, approximately 5,000 to 6,000 different diatom taxa have been identified, with high variations in local species richness among communities (Eloranta et al. 2007, Soininen & Heino 2007). Even at the scale of a single sediment sample, hundreds of species can be identified (Dixit et al. 1992).

3.1. Biology and morphology

Diatoms are present worldwide in all saline, brackish and freshwater standing and running waters throughout a year (Dixit et al. 1992). In freshwaters, diatoms are found from rivers and lakes to the smallest ponds and periodically dry wetlands (Stevenson 1996). Due to high local abundance and consequently high species richness and population densities, diatoms constitute a large proportion of biodiversity in freshwaters (Martiny et al. 2006, Soininen 2007, Hillebrand et al. 2001). In the benthos, diatoms may form majority of the community biomass and both functional and species diversity (Lowe 1996, Kalff 2002, Wetzel et al. 2012). In lentic waters, diatoms are found from nearly every available microhabitat, forming distinct communities according to various substratum types (Dixit et al. 1992). Planktic diatoms live suspended in the water column and move freely with currents, while benthic species live attached to or freely on or in the substratum at the bottom of the water column. The distinction between plankton and benthos is not straightforward, though, as some more mobile species spend their lives traveling between the upper and lower water layers (Round et al. 1990). The attached benthic diatoms are further divided into different ecological groups according to the substratum they grow on. Periphytic species grow on hardy substrata such as wood, plant or rock (Stevenson

1996). The latter gives name to *epilithon* – species growing on varying rocky surfaces (Eloranta et al. 2007). The best developed epilithic communities are found from completely and continuously submerged surfaces. Although more diverse both in terms of species abundance and life forms, the ecological knowledge of benthic diatoms still lacks behind that of planktic species (Round et al. 1990).

Diatom life cycles are short, allowing rapid genetic differentiation and growth in abundance while environmental conditions are optimal (Martiny et al. 2006, Gudmundsdottir et al. 2013). For most of the diatom species, generation times are usually counted in days (Hillebrand et al. 2010). Most of the diatom growth takes place in early spring and autumn. In spring, increasing rates of solar energy enhances photosynthetic activity and thus primary production, leading to fastening biomass growth (Kalff 2002). Due to high diversity, short generation times and species-specific sensitivity to environmental alterations, reactions to environmental fluctuations in water quality are relatively fast, making diatoms are overwhelmingly representative of water physiochemical condition and habitat variability over multiple scales and large geographical extents (Dixit et al. 1992, Lowe & Pan 1996, Gudmundsdottir et al. 2013, Mangadze et al. 2016). Since the rocky surfaces of epilithon themselves do not release chemical compounds to the environment, non-motile diatoms take the needed substances directly from the surrounding water and are thus heavily influenced by the water quality of their habitat (Eloranta et al. 2007). Several diatom-based indices assessing water quality have been developed over recent decades, nowadays laying the base for water biomonitoring, which is a fast and cost-effective way of assessing the environmental condition and water quality by biological indicator species (Dixit et al. 1992, Mangadze et al. 2016).

The morphology of diatoms is diverse, and species may have either unicellular, colonial or filamentous life forms or a combination of some of the three (Stevenson 1996). Based on morphological differences in the valve structure, diatoms are roughly divided into two groups, the pennates and the centrales. Pennate diatoms are elongate, often bipolar and bilaterally symmetrical species making up majority of diatoms, whilst centric diatoms are circular and radially symmetrical. The pennates are mostly benthic species, while centric diatoms are nearly always planktic. The most characteristic feature of diatoms is their resistant cell wall heavy in silicon (Round et al. 1990). The wall consists of two separate units, or valves, linked together by a group of thinner belt-like elements, the girdle bands. Together, these components are called the frustule, which forms the cell external skeleton resistant of decaying (Dixit et al. 1992, Kalff 2002). The frustule can be separated to two halves by the age of the valves and adjacent girdle

bands: the upper part of the cell with the younger valve (the hypovalve) and attached girdle bands is called the hypotheca, while the older valve (the epivalve) and girdle bands are called the epitheca (Fig. 1). A special characteristic of many pennates is the raphe system: usually two longitudinal slits crossing trough the valve along its midline and often separated by a thicker cilica-rich central nodule (Round et al. 1990).

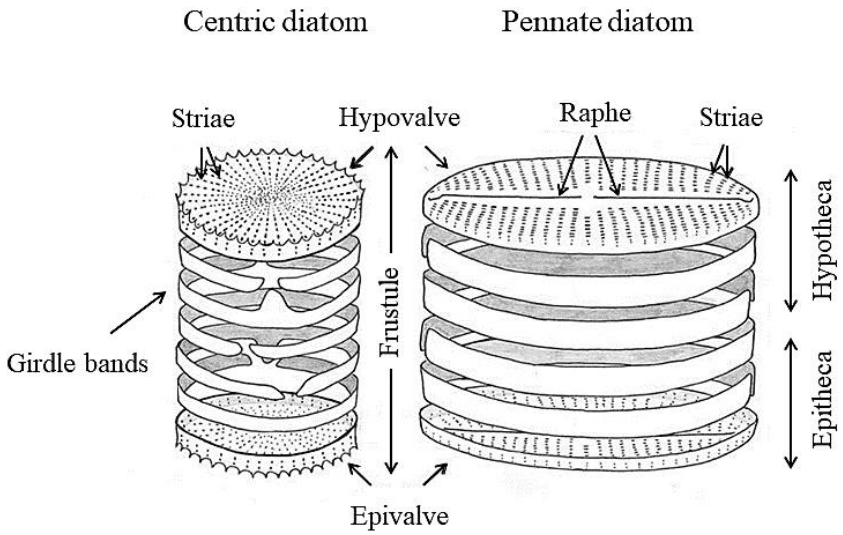


Figure 1. A schematic drawing of centric (left) and pennate (right) diatom cells. The cell, or frustule, consists of upper and lower valve (the hypovalve and epivalve, respectively) and band-like girdle elements linking the upper half of the cell, the hypotheca, to the lower half, the epitheca. The hypovalve is ornamented with thin striae; pennate cells also have a longitudinal raphe system with a central node on their upper valve. Modified after Cox (2014).

The growth of a diatom cell occurs unidirectionally by adding new girdle bands between the two valves, moving the hypotheca and epitheca vertically apart from each other (Round et al. 1990). The upper valve is always smaller in size compared to the lower valve, overlapping the edge of the latter. Thus, the addition of new girdle bands due to cell growth eventually leads to intra-specific variation in morphology, represented by a progressive reduction in the mean cell size and a simplification of the cell shape by each cell division (Mann 1989). The size of the valves thus varies widely among species. Changing environmental conditions may result in inter-specific size differences as well (Snoeijs et al. 2002).

Identification and taxonomy of diatom species has traditionally been based on phenotypic morphological differences in their highly differentiated and uniquely ornamented siliceous cell wall (Dixit et al. 1992) (Fig. 2). The often genera-specific position and structure of the raphe serves as an essential tool in identifying pennate species (Round et al. 1990). Following

developments in species genetics and molecular methods, the number of species in different genera will likely decrease in the future, as morphologically distinct species are united based on their genetic relatedness. This is especially true with centric diatoms, which are extremely difficult to distinguish solely by morphology (Van Dam et al. 1994). Advanced identifications methods such as PCR-based gel electrophoresis and cloning of eukaryotic 18S rDNA gene sequences have also led to taxonomic reclassifications with several changes and updates in diatom nomenclature (Hillebrand et al. 2001, Savin et al. 2004).

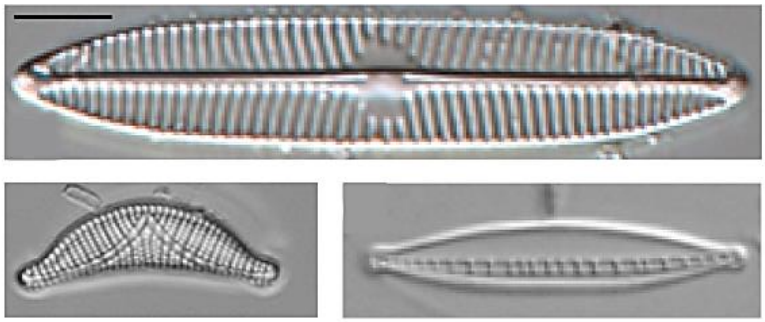


Figure 2. Three common diatom cells seen through a light microscope: *Navicula tripunctata* (top), *Epithemia sorex* (lower left) and *Nitzschia dissipata* (lower right). The length of the scale bar on the upper right corner in the figure is 10 μm. Modified after Szabó et al. (2017).

3.2. Ecology

The ecology of microbial diatoms is nowadays exceptionally well understood (Eloranta et al. 2007). A key characteristic of diatoms is their sensitivity to several environmental stressors. As autotrophic species responsible for primary production, photosynthesis and chemical cycles such as carbon fixation and oxygen generation (Dixit et al. 1992, Stevenson 1996), the populations growth of diatoms is largely limited by several abiotic and biotic environmental characteristics such as water physiochemistry, environmental disturbance events, light availability and biotic interactions. These affect not only the physiology of diatoms, but abundance, diversity and community composition as well (Hill 1996, Heino et al. 2010). Water pH, temperature, dissolved oxygen content and nutrient and ion concentrations are among the most important environmental factors contributing to diatom assemblages (Potapova & Charles 2002, Soininen et al. 2004, Soininen 2007, Bottin et al. 2014). At a genus level, preferences for environmental conditions may vary widely (Dixit et al. 1992), but the species-specific narrow environmental optima and preferences make these microorganisms typical of certain types of habitat, with dominating species being best adopted to the current environmental conditions

(Eloranta et al. 2007, Vilmi et al. 2015). The diatom cell size often determines species-specific responses to environmental conditions, with larger-sized species being more specialized and thus reacting more strongly to certain environmental factors (Snoeijs et al. 2002).

3.2.1. Abiotic environmental constraints

Water conductivity – loosely defined as the capacity of water to conduct electrical current – is one of the most influential chemical characteristics in structuring periphytic diatom communities (Soininen et al. 2004, Martiny et al. 2006, Soininen 2007, Schröder et al. 2015). Conductivity is often translated to water salinity, which describes the total concentration of dissolved inorganic ions in water. Diatoms exhibit a broad range of species-specific salinity tolerances. Salinity gradient roughly divides diatom species to three ecological groups of marine, brackish and freshwater species according to their species-specific tolerances against saline water. The species diversity and distribution of diatoms is also strongly affected by water pH levels, which regulates several physiological cellular processes (Petersen 1996, Weckström et al. 1997, Soininen 2007, Soininen et al. 2016). The relationship between water pH and diatom species richness follows a unimodal trend, with highest diversities recorded in nearly neutral waters. Alkaline waters are usually less fatal than acidic for benthic diatoms (Pither & Aarssen 2005, Eloranta et al. 2007). Species insensitive to water pH alterations are extremely rare: even wide species-specific tolerance ranges have borders at certain levels of the pH spectrum (Van Dam et al. 1994).

Nutrient concentrations are among the most important factors contributing to phytoplankton occurrence, reflecting the biotic productivity of a community (Soininen et al. 2011, Soininen & Meier 2014). As autotrophic primary producers relying on nutrients for photosynthesis and growth, algal diatoms are directly affected by shifts in water nutrient balance (Mangadze et al. 2016). In water bodies, nutrient concentrations form a vertical gradient, with highest concentrations at the surface water and lowest at the bottom (McGormick 1996). Phosphorus and nitrogen are the most important limiting inorganic nutrients for diatoms (Eloranta et al. 2007); other essential nutrients such as carbon and silicon – the primary constituent of the diatom cell wall – are of far lesser importance (Round et al. 1990, Borchardt 1996). Species- and community-scale responses to variation in water nutrient concentrations is often consistent across multiple spatial scales (Spivak et al. 2011), but changes in diatom species richness vary according to the current trophic state of a habitat. In nutrient-limited oligotrophic waters, positive linear trends have been detected (Soininen & Meier 2014). In nutrient-rich eutrophic

waters, instead, algal responses to nutrient enrichment are somewhat different, with species richness following a slightly skewed unimodal trend, the diversity decreasing until the highest levels of nutrient concentration due to high dominance of only few environmentally adopted species in the community (Eloranta et al. 2007, Soininen & Heino 2007).

The reciprocal importance of phosphorus and nitrogen for diatoms is somewhat geographically structured (Borchardt 1996). In freshwater environments, diatom growth is usually more limited by phosphorus, while marine species tend to be more dependent on nitrogen concentration (Soininen & Meier 2014). However, high levels of nutrient enrichment may shift the pattern towards nitrogen limitation in freshwaters as well (Kalff 2002). For freshwater benthic diatoms, these two nutrients are usually of only short supply and near their limiting concentrations, yet not actually limiting. Communal responds to changing nutrient levels are yet poorly understood, and due to different tolerances against environmental conditions, species-specific locations along water nutrient gradient vary widely. Nutrient uptake by diatoms is closely related to water temperature and light conditions, with enhanced uptake encountered as these conditions shift farther from the species optima (Borchardt 1996).

In the freshwater benthos, water temperature is determined primarily by direct solar radiation, closely following shifts in air temperature. Water temperature has an important role in regulating microbial biochemical reactions and the biochemistry of individual cells, maintaining cell growth, inducting sexual reproduction and in formation of resting spores (DeNicola 1996). Water temperature also affects diatom communities indirectly by changing water physiochemical characteristics (Virta & Soininen 2017). In general, algal species richness exhibit a unimodal relationship with water temperature. The optimal water temperature for microbial algal species is often close to the upper limit of their temperature range, and while some diatom species tend to favor higher temperatures, others thrive in cooler waters (DeNicola 1996, King et al. 2006).

For primary producers, light is a fundamental resource enabling photosynthesis of inorganic compounds into biomass (Hill 1996). As completely submerged primary producers, availability of adequate light is essential for periphytic diatoms, altering not only diatom physiology but community structure as well (Lowe 1996, Kelly et al. 1998). Consequently, the distribution of periphytic diatoms is restricted to the illuminated – i.e. *euphotic* - zone of water column (Eloranta et al. 2007), although clear waters also have their downside for the benthos as deep penetrating UV radiation may damage diatom cells genetically (Hill 1996). Water depth and

habitat area have also been detected as important drivers of variation in diatom communities (Martiny et a. 2006, Soininen 2007, 2012). Although likewise influential, physical environmental factors are nevertheless of lesser importance than environmental ones for diatoms (Soininen & Heino 2007).

3.2.2. Biotic environmental constraints

Despite the overriding importance of environmental variables for diatoms, biotic interactions are likewise important yet less studied (Soininen & Heino 2007). As primary producers and often the most diverse photoautotrophic group in lentic and lotic waters, diatoms are an important – if not a major – part of complex aquatic microbial food webs (Bott 1996, Lamberti 1996, King et al. 2006). Diatom abundance and community composition are regulated by grazing by several larger organisms such as aquatic invertebrates (Eloranta et al. 2007). Some of the diatoms are more resistant to grazing than others, but herbivory almost certainly reduces the biomass, species diversity and primary production, prevents species succession, and affects nutrient concentrations and species composition of a community (Steinman 1996, King et al. 2006). Besides herbivory, competition over limited resources in a community also affects diatoms by reducing biomass of species less capable of successfully compete for the resources (Borchardt 1996, McGormick 1996).

Environmental and physical disturbances stem from the deviation in environmental conditions from the species optima for different factors, disrupting the community of microbial organisms especially prone to frequent small-scale disturbance events (Stevenson 1996, Soininen & Heino 2007). Wave-caused water turbulence, variations in water physiochemical variables and increased grazing pressure, scarcity of light and habitat desiccation are among the commonest causes of disturbance for periphyton (Lowe 1996, Peterson 1996). Species tightly attached to the substratum are more resistant to physical disturbance than more loosely attached species. The intensity and frequency of disturbances mostly determines the severity of the community-level outcomes. Low levels of available resources usually serve as an induction for cell dormancy – a metabolic state allowing species survival through unfavorable environmental conditions for cell vegetative growth (McGormick 1996). The dormancy is achieved either by forming morphologically distinct resting spores, or for the most part, by simply transforming the vegetative cell itself to a dormant state. These resting stages are especially vital in aquatic environments prone to desiccation, such as small coastal water bodies on a rocky bedrock (McQuoid et al. 2002). Dense and heavy siliceous cell wall makes especially the largest diatom

species prone to sinking and eventually to sedimentation (Kalff 2002). Due to the resistant cell wall, the sedimented cells form a seed bank in which the cells survive viable for years – even decades – while maintaining diversity through droughts (Dixit et al. 1992, McQuoid et al. 2002). The release of the cells to the water column is promoted by enhanced environmental conditions (Round et al. 1990), following rapid recolonization of the cells leading to notable and long-term shifts in species composition in a community by re-introduction of consecutively germinating species absent from the current assemblage (Kalff 2002, Brock et al. 2003).

3.3. Distribution patterns

Apart from few diatoms capable of rotational movement, diatoms are nonmotile organisms relying mainly on passive dispersal for immigration (Round et al. 1990). As deeply submerged algal group, benthic diatoms are less exposed to wind and wave action and water turbidity compared to planktic species closer to water surface (Virta & Soininen 2017). Despite their sheltered location in the water column, passive dispersal is efficient and occurs frequently via biotic and abiotic dispersal vectors such as wind, water, animals or humans (Soininen 2007, Vanormelingen et al. 2008b).

A deeply rooted view of unicellular diatom distribution ranges states that as passively dispersing species with unlimited long-distance dispersal and often narrow environmental preferences, communities of microbial diatoms would be solely controlled by large-scale stochastic events or local environmental factors. This – accompanied with effective species dispersal – would eventually lead to worldwide distributions over multiple biogeographical regions with no real habitat borders, making diatoms ubiquitous on a global scale (Finlay 2002, Soininen et al. 2004). This pattern is probably best described by Baas-Becking (1934), who developed the hypothesis of microbial distributions, stating '*Everything is everywhere, but the environment selects*.' With no geographical limitations, microbes should thus be capable living wherever their required environmental preferences are met. The *cosmopolitanism* – i.e. unrestricted dispersal accompanied with high species abundancies independent of evolutionary or spatial limitation – of diatoms and other microbial species has been described in detail by numerous former studies (Fenchel & Finlay 2004, Beisner et al. 2006). Cosmopolitanism has been stated to exist among all free-living bacteria, archaea, protists and eukaryotic species smaller than 1 mm – the definition of a microorganism (Finlay 2002). This kind of unrestricted distribution would eventually result in a single, purely environmentally restricted metapopulation with no clear biogeographical or macroecological patterns (Fenchel & Finlay

2004, Martiny et al. 2006). Several microbial characteristics such as extremely high abundance, large populations, good dispersal ability, slow speciation rate, low endemism and low risk of extinction support the view of unlimited random dispersal (Finlay 2002, Green & Bohannan 2007).

Regardless of the traditional view of diatoms being mostly under environmental control, an intense research of diatom distribution patterns has revealed the dependence of these and other microbial species on large-scale climatic, evolutionary and dispersal-related factors as well, resulting in distance decay patterns and regional segregation among communities restricted to distinct habitats (Hillebrand et al. 2001, Soininen et al. 2004, Martiny et al. 2006, Soininen 2012). Consequently, the cosmopolitan distribution pattern is nowadays challenged by numerous study results indicating a strong spatial control over diatom species similar to macro-organisms and quite independent of the habitat type and temporal or spatial scale in question (Potapova & Charles 2002, Soininen 2007, Vyverman et al. 2007, Vanormelingen et al. 2008b, Verleyen et al. 2009, Heino et al. 2010, Astorga et al. 2012, Bottin et al. 2014). Highly endemic species with extremely regionally restricted distributions have been found from diatom communities, contradicting the cosmopolitan nature of microbial species (Mann & Droop 1996, Potapova & Charles 2002). Whilst some or even majority of diatom species do exhibit global distribution ranges, many others do not, adding to the endemism of these microbial organisms (Martiny et al. 2006).

4. ROCK POOLS

Rock pools are geological formations occurring as ubiquitously scattered depressions on rocky outcrops typically surrounded by sea. Despite small size, these physically and biologically diverse waterbodies form disproportionately large share of regional-scale continental aquatic area and biodiversity (Blaustein & Schwartz 2001, De Meester et al. 2005, Downing 2010, Brendonck et al. 2016). Rock pool communities are mainly influenced by habitat isolation, frequent environmental disturbances and intense biotic interactions, resulting in highly specialized, both environmentally and spatially limited biota with good dispersal capabilities under significant consumer pressure (Urban 2004, Vanschoenwinkel et al. 2013). The relative importance of these three community-scale factors for species assemblages depends on habitat duration of a given rock pool habitat (Schneider & Frost 1996).

4.1. Geology and morphology

Rock pools typically occur as a cluster of small and shallow depressions on rocky outcrops, i.e. inselbergs. These isolated, dome-shaped rocky hills cover 15% of Earth's land surface and are often dotted with rock pools (Jocque et al. 2006). Rock pools can be roughly divided to three categories by the rock type of the bedrock they are most often formed on. Erosion and weathering are the leading natural forces in shaping and gouging the underlying rock to water-filled depressions (Jocque et al. 2010, Brendonck et al. 2010, 2016). Majority of the pools are granitic, formed on a robust bedrock of intrusive igneous minerals, which solidify deep in the Earth's mantle under high pressure and temperature. Less resistant minerals are eroded from the bedrock, leaving behind an exposed depression of more resistant potassium feldspar and quartz minerals, forming the granitic base to a pool later to be filled by water. Frost weathering typical of cooler climate of higher latitudes may further crack the pool basin, leaving it prone to corrosion by acidic water covering sediments and detritus on the pool bottom. The age of these persistent granitic pools easily exceeds millions of years, making them one the oldest freshwater systems and least prone to erosion among the rock pool family (Jocque et al. 2010, Brendonck et al. 2016). In alpine and arctic areas, glaciers act as a special force occasionally shaping the formation of granitic rock pools (Meier & Soininen 2014).

Due to similar geological processes responsible for the origin of rock pools, pool morphology is usually simple and comparable between different pool formations (Brendonck et al. 2010). Despite this geomorphological similarity and high physical homogeneity, the shape and size of the pools varies by both basin depth and pool surface area (Hulsmans et al. 2008, Firth et al. 2014). The horizontal shape of granitic rock pools may be variable, but basin structure is usually simple and homogeneous, with concave bottom and gently sloped pool walls reminding the shape of a pan (Brendonck et al. 2016, Häggqvist & Lindholm 2016). Rock pool morphology has its implications for pool biota, determining various environmental conditions in pools. Rock pools are generally shallow, with basin depths often ranging between 5 cm and 30 cm, although deepest pools may reach a depth of 2 m (Brendonck et al. 2010, Naselli-Flores & Barone 2012) (Fig. 1). The pool depth is not a stable variable, however, as it may vary by several centimeters due to changes in precipitation patterns and other local climatic conditions. Unlike other morphological features, pool depth is often a critical factor in determining rock pool biodiversity and community composition (Metaxas & Scheibling 1996, Williams et al. 2004, Firth et al. 2014). The area of rock pools usually varies from less than one to few thousand square meters (Häggqvist & Lindholm 2016, Castillo-Escrivà et al. 2017). Pool volume may

range anywhere between few centiliters and hundreds of liters, with mean volume often somewhere near ten liters (Metaxas & Scheibling 1996, Wilbur 1997, Castillo-Escrivà et al. 2017). Especially in higher latitudes with seasonal variations in rainfall, evaporation and the rate of snow melt, temporal patterns in pool volume are typically highly variable. Water volume affects the desiccation risk of a pool, which strongly impacts species occurrence and persistence in the pool habitat (Altermatt et al. 2009).

Figure 1. Granitic rock pools on the coast of northern Baltic Sea on Pihlajasaari island off the south coast of Helsinki in May 2016. a) A small rock pool with clearly visible granitic base and a light layer of sediments. The depth of the pool is c. 30 cm. b) A larger rock pool housing multiple boulders. Horizontal stripes on the rocks near the water surface are a sign of former water level lowered by evaporation. The vertical length of the pool in the picture is c. 3 m. Photos by author.

4.2. Hydrology

By hydrogeology, rock pools can be considered as closed systems. Once filled, pools hold their water until the end of the wet phase, without any leakage of the water to the environment through impermeable pool basin (Jocque et al. 2010). Water is lost only by evaporation. The only sources of water are rainfall, and to a lesser extent the sea, in the case of coastal pools. Some water may also arrive by unidirectional overflows through watercourses from other aquatic systems such as nearby lakes and rivers or from neighboring interconnected pools, occasionally resulting in thorough exchange of pool water (Jocque et al. 2006, Brendonck et al. 2016, Pellowe-Wagstaff & Simonis 2014). Additionally, ground water may serve as a water source in rare occasions due to capillary forces (Boix et al. 2001). Thus, the basic pool hydrology is typically simple, with little or no variation among pools located in different parts of the world – yet temporal and spatial variations in local pool hydrology may be clear (Hulsmans et al. 2008, Brendonck et al. 2010).

Majority of rock pools are located in coastal areas in immediate adjacency to seawater. Minority of pools, however, can be found from inland areas far away from other natural water bodies. These *freshwater pools* are mostly filled by rainfall, melting snow or ice and ground water (Blaustein & Schwartz 2001, Jocque et al. 2010). Some of the water may enter as a surface outflow from surrounding vegetation or water bodies. Surrounding landmasses block the way to seawater, making these pools suitable habitats for freshwater biota (Metaxas & Scheibling 1993). Water salinity is low, although evaporation may occasionally increase salinities. Contrary to freshwater pools, *marine pools* are located in direct influence of seas. Salinity of the pool water depends not only on the horizontal distance to the sea but is also determined by the vertical location of the pools in relation to the sea surface (Underwood & Skilleter 1996). Marine pools are heavily influenced by the saline seawater, either by direct wave-caused inflows and indirect wind-caused salty splash and sprays, or by periodical tidal cycle. Pool biota is marine, adapted to high water salinities (Metaxas & Scheibling 1993, Therriault & Kolasa 2000, Boix et al. 2001). The location of the pools in relation to the sea surface determines the extent to which pools are regarded as freshwater or marine. Farther from the shore, the influence of seawater decreases, lowering the pool salinity and changing the biota toward rainfall-fed freshwater species. These *brackish-watered pools* house a mixture of freshwater and marine species, with a decreasing share of marine species along the distance gradient. Through wind-induced waves and salty sprays from the sea, marine water extends its influence far away from the shore at best. Some of these pools are located on the coast and archipelago of brackish-watered seas, with moderate water salinity clearly lower than that of larger oceans (Jocque et al. 2010). The water mostly comes from rains with a minor influence of the surrounding sea water (Metaxas & Scheibling 1993, Häggqvist & Lindholm 2016).

Rock pool hydrology is mostly determined by the duration of the aquatic phase. *Hydroperiod*, also referred as inundation period, is a variable determining the average length of the time a pool is filled with water (Hulsmans et al. 2008). Pool hydroperiod reflects the duration of a pool hydrocycle and consists of three separate phases, beginning from the filling of the pool and lasting through the aquatic phase until complete desiccation (Therriault & Kolasa 2001). Pool *hydroregime* is a rooftop term for pool hydrology and its variation in length, timing, frequency and seasonality (Brendonck et al. 2016). Some pools stay continuously inundated as a permanent aquatic environment. These pools – sometimes defined as *ponds* − are usually large and deep. Majority of rock pools are temporary, periodically filling with water before drying out after varying length of time. In higher latitudes, hydroperiod may alternatively be

terminated to pool freezing due to dropping air temperatures (Williams 1996, Hulsmans et al. 2008, Brendonck et al. 2010). Temporary waters may further be divided to intermittent and episodic on the temporal predictability of their wet phase. The former group consists of majority of pools with more predictable – often seasonal − hydroperiods, while the latter is characterized by less regular inundations (Williams 1997). Seasonal changes in rainfall, temperature and wind conditions regulate the hydrology and duration of the wet phase of these pools.

Pool desiccation frequency depends directly on local evaporation rates and precipitation patterns, with large interannual differences in pool hydroperiod (Boix et al. 2001, Hulsmans et al. 2008, Altermatt et al. 2009). The temporal scale of hydroperiod duration extends not only from seasons to years but from days and weeks, strongly affecting the hydrological environment of rock pools (Metaxas & Scheibling 1996, Jocque et al. 2010, Naselli-Flores & Barone 2012, Häggqvist & Lindholm 2015). Variations in hydroregime are often more pronounced in deeper pools, which are capable of retaining various water levels along the depth gradient (Vanschoenwinkel et al. 2009). Hydroperiod is the most important single factor affecting species occurrence, diversity and colonization in rock pools, strongly altering species richness and community composition by selecting species with suitable adaptations and life history strategies to a given pool environment (Williams 1996, Schneider & Frost 1998, Boix et al. 2001, Hulsmans et al. 2008, Vanschoenwinkel et al. 2010, Brendonck et al. 2016).

4.3. Environmental characteristics

4.3.1. Abiotic and biotic environment

Despite similar morphology and hydrology, water physiochemical characteristics are usually spatially variable, resulting in high environmental heterogeneity among individual pools (Metaxas & Scheibling 1996, Meier & Soininen 2014, Häggqvist & Lindholm 2015). Highly heterogeneous and patchy physicochemical conditions create occasionally vertically stratified microhabitats in the water column (Häggqvist & Lindholm 2016). Pool hydroperiod has several direct and indirect impacts on pool water physiochemistry (Schneider & Frost 1996, Naselli-Flores & Barone 2012, Häggqvist & Lindholm 2015). For this reason, several water physiochemical variables, such as water pH, conductivity and temperature, along with dissolved oxygen and nutrient concentrations, vary along the hydroperiod gradient, with extreme values recorded at the beginning or at the end of the inundation period when pool volumes are at the lowest (Brendonck et al. 2010, Firth et al. 2014). As the pool fills by rainfall at the beginning of the hydroperiod, the sudden large amount of water diffuses chemical

compounds and other particles to fit the larger pool volume. This can be observed as diluted water with low nutrient and high dissolved oxygen concentrations, low conductivities and salinities, neutral water pH and cooler water temperatures. Conversely, when water becomes scarce due to pool drying toward the end of the hydroperiod, chemical compounds are concentrated into a smaller space, enriching the water (Jocque et al. 2010). Evaporating water gives way to higher nutrient contents and conductivities, more acidic and less oxygenated water with a higher salinity and rising water temperatures (Williams 1996, Therriault & Kolasa 2001, Naselli-Flores et al. 2015, O'Neill 2016).

Lowest water conductivities are measured during the highest water level. At the start of the hydroperiod, water conductivities may stay below 10 μS/cm, increasing by hundreds of units as the pool volume decreases toward the end of the hydroperiod (Brendonck et al. 2016). Among granitic rock pools, conductivities are often highly variable and may range from at least 2 μS/cm up to 20,200 μS/cm (Jocque et al. 2010, Székely & Langenheder 2013). Water conductivity is also affected by pool proximity to sea regulating the entry of saline seawater. In brackish-watered rock pools, the influence of seawater may create vertical salinity gradients (Metaxas & Scheibling 1993), but the shallow nature of most of rock pools usually prevents this stratification from occurring.

Pool water temperature follows air temperature by a somewhat constant manner (Brendonck et al. 2016). Water temperature usually correlates positively with the amount of UV radiation and negatively with wind speed, evaporation rate, the amount of rainfall and pool depth (Hill 1996, Martins et al. 2007, Jocque et al. 2010, Naselli-Flores & Barone 2012, Song et al. 2013). In coastal rock pools, wave action lowers temperatures by cooling the water via turbidity and salty splashes (Metaxas & Scheibling 1993, 1996). Pool volume affects water temperatures, since is larger water amount is less easily heated by solar radiation. Temporal fluctuations in water temperature may be high, widening the temperature range from that of deeper waters (DeNicola 1996, Williams 1996). Depending on local climatic conditions and the geographical location of a pool, water temperature may range from a freezing point up to 40 °C (Jocque et al. 2010, Brendonck et al. 2016). A special characteristic of rock pool habitats is thermal stratification of pool water. This vertical gradient is due to seasonal and diurnal temperature differences in the shallow water created by inequal amounts of solar radiation between upper and lower water layers of the pool. Cooler and denser water deeper in the pool prevents these water layers from mixing, resulting in thermally stratified environment (Ganning 1971, Dethier 1984). Deeper pools are usually more strongly thermally stratified than shallower ones (Martins et al. 2007).

Thermal stratification creates chemically variable microhabitats for microscopic pool species (Häggqvist & Lindholm 2016). Water transparency, strong winds and heavy rains enhance vertical mixing of the water column.

Although abundant in light sufficient for primary production, in shallow freshwater rock pools nutrient levels are generally low, reflecting an oligotrophic environment (Fairchild et al. 2005, Jocque et al. 2010). However, the relative nutrient concentrations in ephemeral waters may be higher compared to those of more permanent ones (Naselli-Flores & Barone 2012). As mostly autotrophic habitats, nutrients in rock pools are mainly accumulated via decaying organisms, vertebrate fecal inputs and organic material from the external terrestrial vegetation (Lamberti 1996, Methratta 2004, Brendonck et al. 2016), which is reflected especially in pool nitrogen concentration (Ganning & Wulff 1969, Osborne & McLachlan 1985). Nutrients may also be released from pool bottom through resuspension of sediments via bioturbation by aquatic fauna (Fairchild et al. 2005). High levels of photosynthesis rise water pH, which further increases nutrient release from pool sediments (Bradshaw et al. 2002). Removal of nutrients from pools mainly happens via rainfall-induced flushing and wind erosion carrying away pool sediment particles. Terrestrial predators may decrease the nutrient load by removing organisms from pools, and occasionally nutrients may be lost by emigrating pool inhabitants (Wilbur 1997, Brendonck et al. 2016). Nutrients are also removed via uptake by pool algae and macrophytes and denitrification processes by bacteria (Metaxas & Scheibling 1996, Fairchild et al. 2005). Due to close contact to both terrestrial inland areas and the adjacent sea and their nutrient resources, coastal rock pools tend to be slightly more eutrophicated than more inland pools (Methratta 2004).

For pool biota, nitrogen and phosphorus are probably the most valuable nutrients (Soininen & Meier 2014). Dissolved nitrogen and phosphorus concentrations are usually at the highest in the beginning of the hydroperiod, when the biota of the recently-filled pool is yet to stabilize itself and nutrient uptake is thus reduced. Total phosphorus concentration in coastal rock pools typically range from less than 5 μg/L to close to 100 μg/L, while concentrations of total nitrogen may range from few hundred to nearly 2,000 μg/L (Häggqvist & Lindholm 2016). Spatial differences in pool nutrient contents are usually high, exceeding the variability encountered in larger waterbodies (Søndergaard et al. 2005, Meier & Soininen 2014, Häggqvist & Lindholm 2015). During calm weather, vertical stratification patterns in nutrient concentrations may occasionally arise with higher concentrations of especially phosphorus found from the pool bottom (Song et al. 2013, Häggqvist & Lindholm 2016).

Water pH follows a diurnal cycle, with lowest values generally recorded in the morning (Brendonck et al. 2010). Water pH may vary anywhere between at least 4.0 and 11.0 (Jocque et al. 2010). Active photosynthesis rises pH levels (Bradshaw et al. 2002, Vanormelingen et al. 2008a); water pH is also affected by the current phase of pool hydroperiod and inflow of saline water, the latter decreasing pH levels. Despite high temporal fluctuations, vertical differences in water pH are usually small (Häggqvist & Lindholm 2016). Among other pool physiochemical variables, water dissolved oxygen concentrations largely vary both diurnally and seasonally, with lower oxygen levels during the night due to weaker photosynthetic activity (Metaxas & Scheibling 1993, Brendonck et al. 2016). As shallow and often sparsely vegetated environments, rock pools are generally abundant in light, allowing UV radiation to easily penetrate directly to the pool base (Hill 1996, Williams 1996, Häggqvist & Lindholm 2015).

Although simple by food web structure, local biotic interactions such as predation, herbivory and competition strongly affect rock pool communities (Metaxas & Scheibling 1993, Jocque et al. 2010, Soininen & Meier 2014). The relationship between biotic and abiotic processes and the occurrence and strength of the successional phases change along pool hydroperiod and permanence gradient. In short-lived, highly temporal pools, the wet phase is too short for predators to hatch in or immigrate to the pool in large quantities (Schneider & Frost 1996, Boix et al. 2001). In the absence of harvesting pressure caused by predators, these communities – interrupted by premature pool desiccation – stay mostly under control of local abiotic factors. However, as the wet phase proceeds or pool permanence increases, rising water volume suppresses the severity of environmental disturbances and offers more space for biotic interactions to take the control over abiotic factors (Therriault & Kolasa 2001, De Meester et al. 2005, Jocque et al. 2010).

4.3.2. Environmental disturbances

Aquatic systems are prone to environmental disturbances. Rock pools are usually small and shallow (Jocque et al. 2010). The buffering capacity against environmental stressors hence is lowered, strengthening the effects of changing physiochemical conditions on pool biota (Ganning 1971, Metaxas & Scheibling 1993, Martins et al. 2007, Häggqvist & Lindholm 2015). Common environmental disturbances in rock pool systems include pool desiccation, wind-caused water turbulence, excessive predatory pressure and alterations in water physiochemistry such as water temperature, pH or exposure to solar radiation and salinification from seawater inputs (Dethier 1984). Pool desiccation is the severest form of hydrological disturbance

(Williams et al. 2004, Vanschoenwinkel et al. 2013). Frequent disturbances promote community disassembly in the form of reducing microhabitats, enhancing biotic interactions, disrupting species life cycles, inducing cell dormancy and increasing emigration rates (O'Neill 2016), resulting in unstable communities (Dethier 1984, Therriault & Kolasa 2000, 2001, Jocque et al. 2006, 2007, Altermatt et al. 2009).

As ephemeral habitats, changes in environmental conditions require special physical and morphological adaptations for survival (Dethier 1984). Especially permanent pool species and passive dispersers experiencing the unfavorable conditions *in situ* or uncapable of independently escaping unfavorable hydrological conditions have developed various traits to survive over the unfavorable conditions (Blaustein & Schwartz 2001, Vanschoenwinkel et al. 2009, Jocque et al. 2010). Natural selection seems to favor species propagules with long dormancies and delayed germination. A typical life history adaptation of pool species is *diapause*, inducing species dormancy lasting until conditions improve again (Williams 1996). Maturation rates are typically fast, with species hatching immediately after pool filling by rainfall (Brendonck et al. 2010). Majority of rock pool species are ecological specialists with high tolerances for environmental stress; dominating generalist species with wide environmental niches are rare (Dethier 1984).

4.4. Spatial connectivity and distribution patterns

Rock pool formations typically occur as hierarchical nested structures, with individual pools forming distinct pool clusters distributed over rocky outcrops dotting the landscape. The number of pools in a cluster may vary from one to one thousand and distance between separate pools from tens of centimeters to few kilometers (De Meester et al. 2005, Brendonck et al. 2010, Castillo-Escrivà et al. 2017). As a mosaic of closely located depressions over small spatial extend, individual rock pools serve as small-scale communities connected by species dispersal to a single metacommunity (Blaustein & Schwartz 2001). Despite connecting waterways in some pool metacommunities, rock pools are always more or less isolated from each other, making them more isolated than aquatic habitats of larger size (Søndergaard et al. 2005). The degree of pool isolation is usually variable but easily quantified (Brendonck et al. 2010). The mean distance from a single pool to neighboring rock pools determines the relative *isolation* of the pool, whilst *connectivity* expresses the number of waterway connections departing from or arriving in the pool in a pool cluster (Meier & Soininen 2014). These two simple measures give an overview of current dispersal dynamics in the rock pool metacommunity at a certain scale.

Together with spatial setting, the dispersal ability of species is the key characteristic determining species communities in fragmented rock pool habitats (Pellowe-Wagstaff & Simonis 2014). Rock pool biota consist of both active and passive dispersers. For passive dispersers, wind and animals are better suited for occasional long-distance dispersal, while water serves as a more continuous and efficient dispersal route (Cottenie et al. 2003, Castillo-Escrivà et al. 2017). Due to the isolated nature of rock pools, long-distance dispersal is thought to be especially limited (Jocque et al. 2010). Occasional precipitation-induced overflows are an important mechanism for passive *hydrochorous* dispersal, serving as an effortless and effective dispersal route for many organisms (Cottenie et al. 2003, Vanschoenwinkel et al. 2007, Pellowe-Wagstaff & Simonis 2014, Castillo-Escrivà et al. 2017). Local precipitation patterns affect the efficiency of water-aided dispersal: the more persistent and less temporally variable the water connection, the more regular dispersal is allowed via the route (Vanschoenwinkel et al. 2008b). In ephemeral rock pools wind has been proven to be the most important dispersal vector for pool biota, maintaining high dispersal rates among local communities. The carrying capacity of wind is mediated by prevailing wind speed and direction, the former being responsible of most wind-aided, or *anemochorous* dispersal. In addition, continuously visiting animals such as water birds and amphibians offer a way for animal-aided *zoochorous* dispersal. Although occasionally intense, zoochorous dispersal rates nevertheless lag behind those of more efficient water- and wind-aided dispersal (Vanschoenwinkel et al. 2008a, 2008b).

4.5. Biodiversity

Rock pool biota is highly diverse, accounting for disproportionately high amount of regional-scale biodiversity (Boix et al. 2001, De Meester et al. 2005, Jocque et al. 2006). Unique biota with several endemic and highly adapted species, high spatial dissimilarities between separate rock pools, high morphological heterogeneity and spatial isolation of the pools are among the major causes of this remarkable species diversity. Species richness and community dissimilarity of these temporary habitats may even exceed that of permanent water bodies, and ephemeral pools have indeed been proven to hold the most diverse biota among rock pools (Boix et al. 2001, Therriault & Kolasa 2001, Jocque et al. 2010).

Rock pool biota can be divided into two contrasting groups by the duration of their occurrence in a pool. Permanent pool fauna consists of specialized species capable of surviving through fluctuating environmental conditions without a need to leave the pool habitat. Temporary fauna, instead, consists of a group of opportunistic and pioneering species occasionally visiting the

pools, changing habitat to more permanent when conditions turn unsuitable (Williams 1996, 1997, Brendonck et al. 2010). Species typically have certain ranges on a pool permanence gradient in which they most likely occur, the proportion of temporary species increasing with pool permanency (Jocque et al. 2007, 2010). Rock pool biota mostly consists of microbial species of bacteria, phytoplankton and zooplankton, and macroscopic invertebrates such as crustaceans and dipteran species (Jocque et al. 2006, Soininen & Meier 2014, Brendonck et al. 2016). The shortness of pool hydroperiod restricts the survival of largest invertebrates and fish (De Meester et al. 2005, Søndergaard et al. 2005). Micro- and macroalgal species often comprise the largest share of community abundance (Metaxas & Scheibling 1993, Naselli-Flores & Barone 2012). Coastal pools are prone to frequent visits of water birds, and amphibians are a common feature of pools in spring (Brendonck et al. 2016). Due to small area and nutrient poor growth basin, aquatic vegetation is usually sparse and patchy, being mostly restricted to micro-scale depressions containing organic soil (Vanschoenwinkel et al. 2008b).

5. STUDY AREA

The study area is located on the southwestern shore of western island of Pihlajasaari (66°68'449"N, 38°40'48"E), approximately 2 km away from the coast of Helsinki (Fig. 1). The island is surrounded by the Gulf of Finland, the northern part of the Baltic Sea. The study area consists of a cluster of 30 from fresh to brackish-watered rock pools on a single granite outcrop covering an area of approximately 1,200 m², extending diagonally from southeast to northwest.

The rock base of the study area is geologically old, dating back to c. 1,900 – 1,800 million years (Lehtinen et al. 1998). The outcrop is of metamorphic origin, consisting of resistant granitic rocks. The dominating rock type on the study area is biotite (Maanmittauslaitos 2018). In general, rock pool formations are highly common on the Baltic coast of southern Finland. Over 10,000 islands surrounding the southern and southwestern coast offer a favorable geological ground for formation of these miniature rocky depressions (Pajunen & Pajunen 2007). The soil on the study area is mainly leptosol (Maanmittauslaitos 2018) – a thin, poorly developed and easily erodible coarse-grained soil type rich in gravel and typical of bare rocky ground (Casermeiro et al. 2004, Komarék et al. 2009, Muñoz-Rojas et al. 2012). The vertical profile of the island is flat. On the study area, the altitude reaches its maximum at 5 m above the sea level, with a relative elevation difference of only 2.5 m. Majority of the studied rock pools are located at or close to the sea surface level (Maanmittauslaitos 2018).

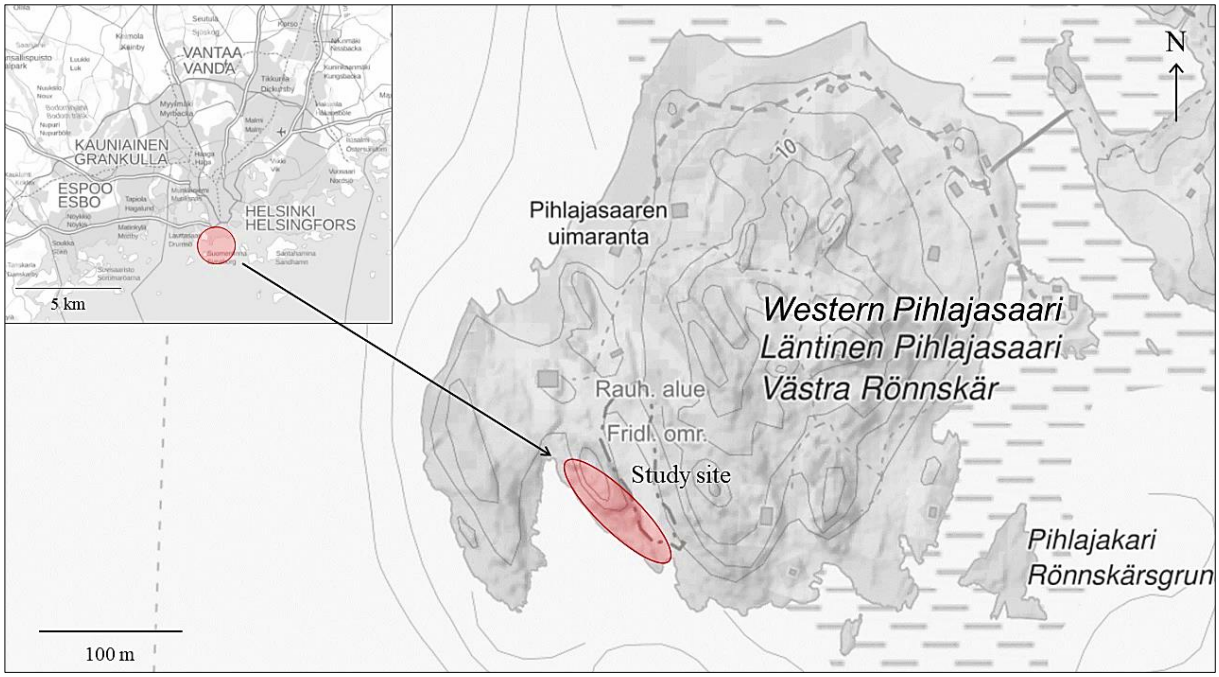
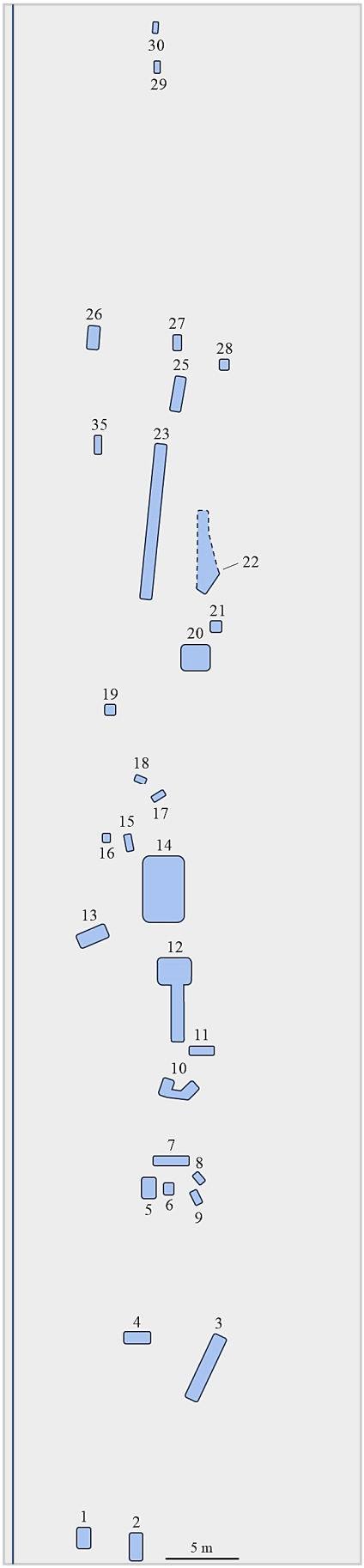


Figure 1. The map of the study area. The study area is located on the western island of Pihlajasaari near the coast of Helsinki (red circle on the index map). The exact study area on the rocky outcrop on southwestern part of the island is shown on the larger map (red oval). The area is c. 1,200 m² by area, extending c. 105 m from northwest to southeast and c. 12 m from northeast to southwest on the island. Map: Maanmittauslaitos (2018).

Pools of varying size and location were randomly chosen for the study. A map of the study area was drawn to a grid paper showing the relative location of the pools to each other and to the seashore (Fig. 2). The scale was consistent with the grid size, one grid covering an area of 2 x 2 m or 4 m² in nature. The studied pools were named by running numbers from 1 to 35 during a visit to the study area a week before the first sampling in May 2016. However, four of the pools dried up until the first sampling: thus, the data do not contain pools 31 to 34. Additionally, pool 24 was merged with pool 22 by a rainfall-induced overflow from June onwards. The merged pools were considered as a single pool numbered 22, and pool 24 was excluded from the data. Thus, the data consists of pools numbered from 1 to 23, 25 to 30 and 35.

The studied rock pools were small and shallow. Low area to volume ratio and the absence of surrounding freshwaters make the pools less prone to sedimentation by runoff and more susceptible to erosion by wind from the sea. Hence, no detectable sedimentation is likely to



occur other than that caused by the accumulation of detritus from the internal load of decaying aquatic flora and fauna of the pools themselves.

The Gulf of Finland serves as a transition zone from eastern fresh waters to more saline water on the western half of the Baltic Sea (Alenius et al. 1998). The water of the nearby Baltic Sea is brackish with an average salinity of 7.4‰ (Meier & Kauker 2003). The average salinity of the surface water is around 5‰ off the coast of Helsinki (Alenius et al. 1998). The studied pools are thus from fresh to brackish-watered, the water salinity forming a gradient between the pools farther away and closer to the seashore. Tidal influence at the northern Baltic area is virtually absent, and the changes of sea level are mainly caused by wind and differences in air pressure (Pajunen & Pajunen 2007).

Majority of the pools are endorheic, relying completely on an outside source – i.e. rainfall – for filling. Only in few occasions at the immediate proximity to the sea can seawater fill the pool from bottom to the surface level; elsewhere, the influence of seawater is too weak to be solely responsible for filling the pool. Pools closest to the sea are thus heavily influenced by the brackish seawater directly entering the pools by wind-caused waves. In addition, during the windiest days of the study period especially in June, pools even meters away from the shore were constantly under the

Figure 2. The map of the studied rock pools based on the grid map of the study area. Shown are all sampled pools numbered from 1 to 23, 25 to 30 and 35 (excluding pool 24 which merged with pool 22 and the dried pools 31-34) and their relative location to each other and to the coastline. The dashed border extending upwards from pool 22 represents the merged area to that pool from June onwards. The seashore is located vertically to the left of the pools, marked as blue solid line. The southern end of the study area terminates to the shoreline as well, while another rocky outcrop and a sandy path borders the upper and right edge of the study area, respectively. The pool closest to the seashore (pool 1) is located 1.6 m away from the sea.

indirect influence of the salty spray from the sea. The pools are filled in spring, some of them drying during the summer. The hydroperiod of this ephemeral environment thus lasts for a few months, being interrupted by occasional drying and terminated by freezing of the water or a total dry-up in the forthcoming winter. Due to the impermeable nature of the granitic pool base, groundwater influence is virtually absent, as is surface outflow from the surroundings in the absence of any nearby abundant vegetation. The pools are isolated from and unconnected to each other, thus forming distinct, clearly delineated aquatic communities (Fig. 3). Some of the pools did overflow because of a heavy rain during the study period, and two of them were merged as a one single pool sharing the water. However, the pools were not permanently interconnected by watercourses at any time during the study period. Despite the disconnectedness of the pools, the small spatial scale of the studied rocky outcrop most likely allows frequent and effective passive dispersal to occur between the pool communities, connecting them as a single metacommunity.

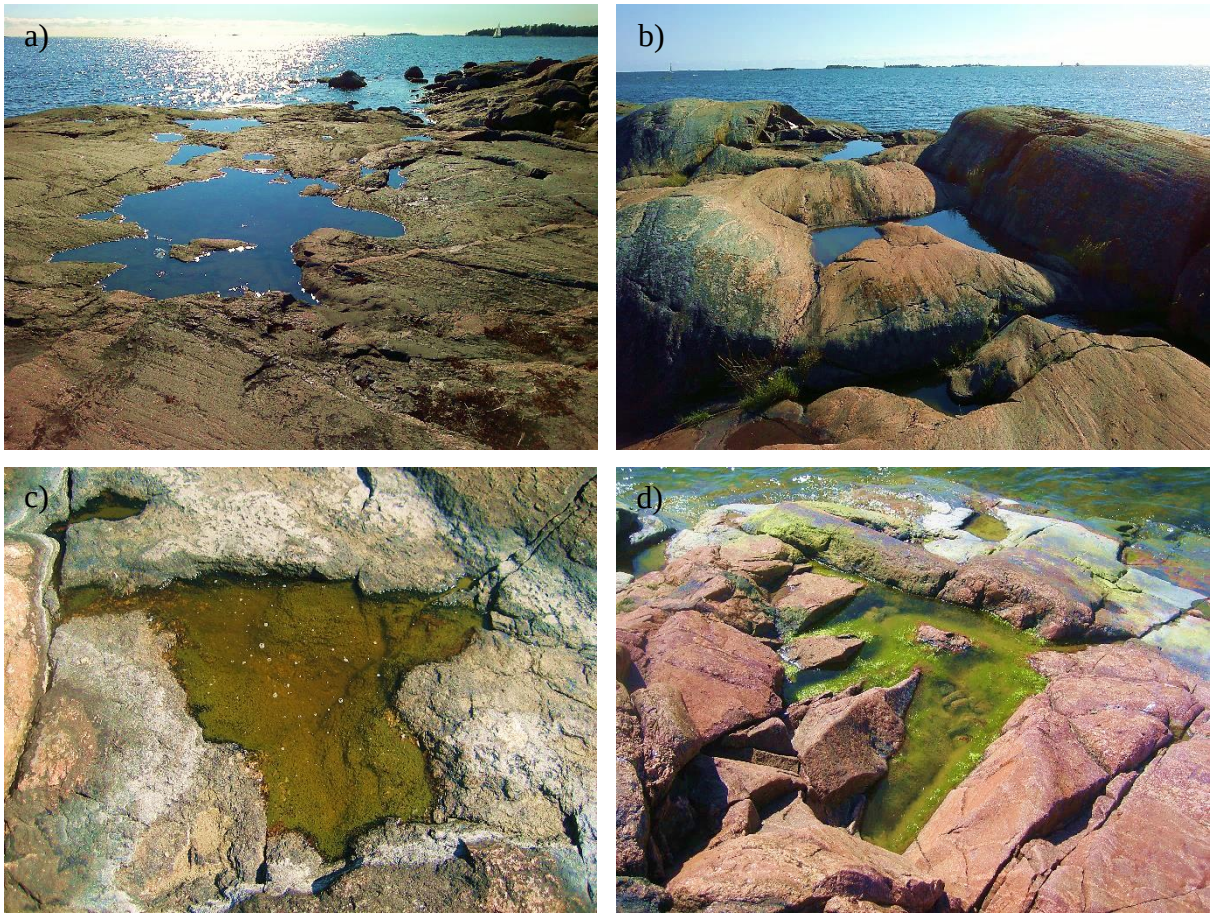


Figure 3. Some of the studied rock pools on the western Pihlajasaari island in the summer of 2015-2016. a) A view from the study area to the northern Gulf of Finland. b) Rock pools facing the southern limit of the study area to the southeast. Some of the studied pools are on the front. c) and d) Studied rock pools of varying sizes. Pool c) is one of the smallest studied pools with an area of approximately 0.3 m², while pool d) is among the larger pools with an area of approximately 4 m². The latter pool is at immediate proximity to the sea and exceptionally highly covered by macroalgae. Photos a) and b) by Anette Teittinen, others by author.

As a part of the southern coast of Finland, the study area belongs to the warm-summer humid continental climate zone of the Köppen climate classification (Köppen 1884). During the 30-year observation period of 1981-2010 by Finnish meteorological institute, the annual daily mean air temperature at the south coast of Finland was 5.8 °C, and the daily mean rainfall 656 mm (Ilmatieteenlaitos 2018). In Helsinki, the annual monthly mean air temperatures ranged from May to July were between 10 °C and 18 °C, and the daily mean rainfall from c. 40 mm in May up to 65 mm in July.

The Pihlajasaari islands belong to a hemiboreal forest vegetation zone together with other southern and southwestern coastal areas of Finland (Westerholm & Raento 1999). The rocky coast itself is rather treeless (Maanmittauslaitos 2018). Because of the harsh nature of the exposed rocky outcrop, terrestrial vegetation on the study area is sparse and low, consisting mainly of occasional grasses, mosses and hays surrounding the pools. Besides of diatoms, the flora and fauna of the pools and nearby rock was not surveyed, but some visual observations were made. In some of the pools, large amounts of tadpoles and larvae of flying insects were detected mainly in May. In addition, water birds such as ducks and seagulls were detected in many of the pools throughout the study period. In minority of the pools, small macroalgal beds were found mainly in June and July. Due to the lack of terrestrial and aquatic vegetation on the area, the pools are not shaded; rather, the UV radiation effortlessly reaches the bottom of the pools, making light conditions comparable for each of the pools.

Nutrient enrichment on the area is likely mostly of faunal and floral origin, caused by decaying terrestrial vegetation such as leaf litter, aquatic insects and their larvae and nutrient inputs from e.g. frogs and tadpoles, bird droppings and other animal feces. The study area is situated near a popular beach during the summer months, but the rocky outcrop is somewhat isolated from the main paths and roads, buildings and attractions on the island. As an unbuilt region by land use (Maanmittauslaitos 2018), the anthropogenic influence for the studied pools may thus be of minor importance, but likely existent. However, due to occasional faunal and anthropogenic influence and the highly eutrophic state of the surrounding Baltic Sea and the polluted Gulf of Finland (Rönnberg & Bonsdorff 2004), the trophic state of the pool water could be estimated as relatively eutrophic as well.

6. DATA AND METHODS

6.1. The data

The field data were collected monthly in three different occasions between on May 17th, June 22nd and July 22nd of 2016. To avoid effects of any diurnal changes on pool physiochemical characteristics, each sampling was timed from early midday to early afternoon. During each sampling diatom and water samples were collected simultaneously from each of the 30 pools. Water samples were always collected before diatom samples to avoid any mixing of water and its chemical compounds caused by diatom sampling. In addition, pool morphometrics and some water physiochemical characteristics were measured in the field. The pools were always sampled in the same order, beginning from the southwestern corner of the study area and proceeding to the northeast along the shoreline.

6.1.1. Environmental data

Environmental data consists of water physiochemical characteristics and pool size measurements. Water physiochemistry was described by water pH, conductivity and temperature, together with total nitrogen and total phosphorus concentrations. Of these physiochemical variables, water pH, conductivity and temperature were measured in the field with YSI field meter. From each pool, a water sample was collected in a 0.5L plastic container for laboratory determination of total P and total N concentration. Water samples were preserved at 4 °C until laboratory analyses. The size of the pool was measured by its maximum length, width and depth with a meter stick to the nearest centimeter. From these morphological measurements, pool area was calculated. Maximum depth was used instead of average depth since the former better describes the duration – i.e. the time pool is filled with water – of each pool habitat (Vanschoenwinkel et al. 2007). All pool morphometrics were converted to meter scale before statistical analyses.

The nutrient concentrations were determined in the laboratory of the Department of geosciences and geography approximately in a week from each sampling event. The determination for total P and total N followed standards SFS-EN ISO 6878 and SFS-EN ISO 11905-1, respectively. The method for assessing nutrient concentrations was based on a closed wet combustion, in which the samples are heated in a closed container called autoclave under high temperature and pressure (Virkanen et al. 2017). In the autoclave, the samples are heated by water vapor, which dissolves organic and inorganic components of the samples. Following the preliminary

treatment of the samples, the nutrient concentrations were measured with UV/VIS spectrometer. The spectrometer registers the amount of radiation absorbed by the molecules or accompanied, colored complex ions of sample solutions on visible light and ultraviolet wave lengths. Total P results were inside the determination limits and thus all acceptable. Determination of total N was occasionally disturbed by organic matter, which like nitrogen, also absorbs radiation on ultraviolet wavelength of the spectrum. To ensure the reliability of total N results, absorption was also checked at a wavelength of 275 nm – the part of the spectrum at which most of the organic matter absorbs radiation. With part of the samples, the absorption at the wavelength of 275 nm exceeded 5.0% of the absorption at the wavelength of 220 nm. With such a high disturbance of organic matter, the reliability of the total N results is violated, and the given values must be rejected (Virkanen et al. 2017). Hence, a total of 21 total N values were rejected: 15 in June and six in July. In addition, results of two samples exceeded the upper total N determination limit in May. These were also rejected from the data.

The rejected monthly nitrogen values were replaced by mean of the values from the two other months, or in the case of four pools with values lacking from at least two months, by mean of three geographically closest pools in each month. The favor of the former method was mostly based on the high spatial heterogeneity often revealed in rock pool nutrient concentrations (Soininen & Meier 2014, Häggqvist & Lindholm 2015). The replacement of the missing values was important since in brackish aquatic environments, the reciprocal role of total P and total N for species as a limiting nutrient is not always clear and as straightforwardly assessed as in completely marine or freshwater systems (Rönnberg & Bonnsdorff 2004, Gudmundsdottir et al. 2013). Thus, replacement of the values enabled the usage of total N in the basic statistical analyses such as in the pairwise correlation tests and spatial autocorrelation. However, the accuracy of the derived values was considered too low for more complicated analysis, the inclusion of the values possibly seriously violating the reliability of the statistical results. Hence, total N records were excluded from all other monthly statistical models. Instead, completely reliable and thus statistically applicable total P records were used as a proxy for pool nutrient concentrations. All total P measurements were converted from mg/L to µg/L before statistical analyses to correspond the scale of total N measurements.

6.1.2. Spatial data

Spatial data consisted of pool X and Y coordinates and mean isolation. The coordinates were estimated from the grid-base map of the study area (for details, see chapter 5), the grid

describing natural distances between pools. The scale of the map was 1:2, one grid on a map corresponding 2 m overland distance in nature. The origin on the map is located at the lower left corner of the study area near the shoreline. The Y coordinates thus describes the horizontal distance of the pools from the origin parallel to the shoreline, while the X coordinates are based on the perpendicular distance of the pools from the shore. Pool distance from seashore strongly influences water physiochemical conditions such as conductivity and temperature through salty sprays and may hence influence species community composition in the studied pools (Ganning 1971). Rock pool isolation have been revealed to play a major role for species dispersal, especially for passive dispersers (Vanschoenwinkel et al. 2008a). The isolation of a pool was calculated as a mean Euclidean distance from a given pool to five geographically closest pools in the pool cluster (i.e. the sum of all nearest distances to all five closest pools divided by the number of the pools in a cluster = 5), following the method presented by Vanschoenwinkel et al. (2007). Since the studied pools were not interconnected by waterways, no measures of pool connectedness were included in the data. All spatial measurements were attained in meters.

6.1.3. Species data

Benthic diatom samples were collected simultaneously with water samples during each sampling event from May to July 2016. In higher water temperatures, communal reactions to water quality are faster (Kelly et al. 1998). Hence, wider scale of composition change can be attained by sampling diatoms during the summer (Eloranta et al. 2007). The sampling mainly followed methods proposed by Eloranta et al. (2007) originally based on EN 13946 standard (2003) for running waters, which is also applicable to lentic systems when slightly modified (King et al. 2006). The standardized sampling methods allow reliable comparing of site-specific species richness (Soininen & Meier 2014). The sampling of the diatom cells was carried out with a toothbrush. A toothbrush has proven to be a useful tool in diatom sampling since damaging the cells by a soft and gentle brush can be minimized (Kelly et al. 1998). Due to high local species diversity typical of diatoms, relatively few samples are usually needed to capture the nature of site-specific community composition (Eloranta 2004). Thus, ten subsamples from an area of 5 x 5 cm were collected from each pool and combined as a single composite sample in a plastic test tube in the field. Pooling samples prevents the effects of small-scale variation caused by physicochemical differences in rock pool microhabitats for sampled communities (King et al. 2006). To increase the probability that the samples contained a sufficient amount of diatom cells for further analysis, each of the brushing was repeated thrice for every single

subsample. Between every brush scrape, the toothbrush was rinsed in pool water to remove any cells attached to the brush. This effectively prevents contaminating between the samples (Kelly et al. 1998).

The samples were collected from the epilithon from the bottom of the pools. Epilithon – i.e. rock surface – is a recommended surface for diatom sampling, since it is physiochemically stable and thus spatially invariable growing surface for species communities. Only in the case of the deepest pools samples were collected from the pool walls deep enough to ensure the frustules were sampled from permanently submerged, non-exposed substrata (Kelly et al. 1998, King et al. 2006). Due to the shallowness of the pools and lack of shading vegetation, the euphotic zone effortlessly extends to the pool base, making light conditions between the pools comparable. This allowed sampling equally around the pool close to or from the pool bottom (Kelly et al. 1998). Deeper in the pool environmental conditions such as light availability, water temperature and nutrient concentrations tend to be more stable and thus more favorable for diatoms to exist (Firth et al. 2014). On average, the samples were collected c. 20 cm below the water surface, which is the standard sampling depth recommended for epilithic diatoms (Eloranta et al. 2007). From the largest pools, samples were collected comprehensively from different sides of the pool to ensure sufficient sampling extent. The collected samples were preserved in a cool and dark place at 4 °C until further treatment to prevent the cells from dividing (Eloranta 2004).

The diatom samples were treated with an open wet combustion in the laboratory of the Department of geosciences and geography in next few weeks from the sampling based on methods recommended by Kelly et al. (1998) and Eloranta (2007). The samples contained in a plastic test tube were heated in water poultice and treated with 30-% Hydrogen peroxide to oxidize the organic content. Next, the samples were washed with distilled water and centrifuged thrice for ten minutes with a speed of 2,000 rounds per minute to remove oxidizing acids. Of each of the 30 samples, one slide was prepared for microscope analysis by attaching a drop of the cleaned suspension pipetted on a coverslip with resin. The samples were left to dry at a room temperature overnight. Lastly, the complete slides were heated on a cooking plate to evaporate the excessive resin. Of the most sparsely celled or otherwise disqualified samples, replicate slides were prepared afterwards to ensure a sufficient number of cells for microscope analysis.

The diatom cells were counted between autumn 2016 and spring 2017 with a light microscope at 1,000 x magnification. From each slide, a minimum of 500 frustules were counted. From two extremely sparsely celled slides, less than 500 frustules could be counted, even with the help of extra slides. In case of broken frustules, the cell was counted if half of the frustule was left identifiable. The cells were counted systematically by proceeding horizontally from left to right and vertically in rows from the top to the bottom of each slide. After reaching an amount of 500 counted frustules, all the remaining uncounted cells were counted from the current view and then the counting finished. Due to this, slightly more than 500 cells were counted from most of the slides. The total, absolute cell abundances were thus converted to relative percentages to make the cell number of different samples comparable with each other. Identification of the diatom cells was based to the species guides of Krammer & Lange-Bertalot (1986-1991) and Lange-Bertalot & Metzeltin (1996). The diatom cells were identified to the lowest taxonomic level possible, i.e. to a species level. In few occasions, identification to species level was not possible due to broken or overlapping cells. Additionally, few – mainly marine – cells remained unidentified. These cells were identified to genus level. The unidentified but intact cells were named after the morphologically closest species they resembled, abbreviated by *cf.* Of one of the species, *Gomphonema exiguum*, two subspecies (*G. exiguum var. exiguum* and *G. exiguum var. minutissimum*) were identified on the basis of their divergent morphology. Since the taxonomy in the species guides was somewhat out of date, the species names were finally updated to correspond current taxonomic nomenclature.

6.2. Statistical methods

Statistical analyses included spatial autocorrelation, generalized linear models, redundancy analysis, variation partitioning and Mantel and partial Mantel tests. Of these analyses, all but spatial autocorrelation belongs to a wide group of multivariate methods widely used in ecological studies, relating multiple species to multiple explanatory variables simultaneously (ter Braak 1986, ter Braak & Verdonschot 1995, Peres-Neto et al. 2006). In ecological data, response variable is often a matrix of multitude of species, which requires separate analyses for each of the species not feasible by a single regression analysis (ter Braak 1987). By multivariate analyses, variation in a single response variable is explained by multiple sets of explanatory variables, allowing reduction and inclusion of wide variety of information in a few statistically meaningful and relevant variables (Schervish 1987, Ranta et al. 2012). For explanatory and response variables, correlation coefficients and basic statistics were calculated. All the analyses

were carried out separately for each month. Additionally, redundancy analysis was also carried out to a dataset combining all the monthly data. All the statistical analyses were done with R statistical software (version 3.4.3) (R Core Team 2018). Spatial autocorrelation was conducted with package *pgirmess* (Giraudoux et al. 2018), and correlation coefficients with package *Hmisc* (Harrell Jr 2018). Redundancy analysis, variation partitioning and Mantel and partial Mantel tests were all performed with *vegan* package (Oksanen et al. 2018).

6.2.1. Statistical pre-processing of the data

Before statistical analyses, the data was tabled with Excel software of Microsoft Office 2016. Monthly records of explanatory environmental and spatial variables and the response variable of species richness were tabled separately in three matrices, variables at the columns and the numbered study sites (i.e. rock pools) at the rows. The monthly species data was likewise tabled, species at the columns and the study pools at the rows. Species abundances were converted to relative percentages to make the by-pool differing abundances comparable. Abundancies were favored over species presence-absence data, since in case of non-uniform species distribution, abundance data is more sensitive to variation in community composition (Beisner et al. 2006). In addition, two separate table sets were created, combining all the monthly environmental and spatial records and relative species abundancies, respectively.

Normality of the explanatory variables and the response variable of species richness were tested with Shapiro-Wilk's normality test (Shapiro & Wilk 1965), which is an effective measure of normality and sensitive for data outliers even for small sample sizes. The normality of a variable is based on the p-value of the test, with significances higher than 0.05 indicating normally distributed observation values. Additionally, observation distributions of each variable were examined with histograms. All the variables that were non-normally distributed (except water pH) were ten-fold logarithmically ($\log_{10}(x)$) transformed to shift the distribution closer to normal. Of all the variables, basic statistics were calculated, and the measured values were plotted for each pool. Values that visually seemed to be unimodally distributed were taken into account in statistical models. Boxplots of some of the environmental variables were created for comparisons of monthly ranges of the records.

Pairwise correlation coefficients and the statistical significance of correlations for every variable were calculated with Spearman's rank correlation coefficient. Spearman's correlation coefficient was used for its special suitability for non-normally distributed variables.

Correlations between variables were also examined with plots. Multicollinearity between a pair of explanatory variables was considered in statistical analyses. Multicollinear variables form a linear relationship, based on which a value of one explanatory variable can be predicted from the value of another variable (Ranta et al. 2012). Strong correlation between variables may hinder the separate effects of the variables on the response variable and add redundant information to the results (O'Brien 2007). With generalized linear models, for example, of a pair of highly collinear explanatory variables both may appear as non-significant in the resulting model irrespective of their individual significance to the response variable (Guisan et al. 2002). Variables exceeding a pairwise correlation of |0.7| were considered as multicollinear and the other variable was always excluded from the model. The exclusion of the variables was done based on their relative importance to the response variable. Of a pair of multicollinear variables, the most relevant for the response variable was chosen to the final models. Due to varying monthly records, different variables were multicollinear in different months. Hence, the monthly models are based on slightly differing explanatory variables.

Of environmental variables, water total P, pH, temperature and conductivity, as well as pool area and depth were included in all monthly models. Pool depth has often been observed as a morphological variable highly responsible for variability in species richness and community composition in rock pools (Martins et al. 2007, Firth et al. 2014). Hence, this variable was selected to the models over pool volume. Since pool depth was also highly multicollinear with pool length and width but not with pool area, the latter was chosen as a measure of pool size. In June, pool Y coordinates were multicollinear with water conductivity. Since conductivity was considered the variable more relevant in explaining species richness, the Y coordinates were left out from the June's model. Due to the difficulties associated with the determination of total N concentration in the laboratory, the validity and reliability of the attained results for this nutrient was questionable at best. Hence, to ensure the reliability of the results of the statistical analyses, only total P was included in each monthly model.

6.2.2. Spatial autocorrelation

The early remark of Tobler (1970) of the greater similarity between geographically close observation values compared to those between more distant ones has given the foundation for the concept of spatial dependence and thus spatial autocorrelation between observation values. In natural systems, *spatial autocorrelation* is an especially common and well understood phenomenon (Legendre 1993). In spatially autocorrelated system – be that for example a mosaic

of small rock pools −, neighboring or nearby observation values are usually not stochastically independent from each other. Instead, values of one variable depend on the values of another variable through space (Fortin et al. 1989). In a positive autocorrelation, neighboring observation values are more similar than could be expected by chance alone. In a negative spatial autocorrelation, instead, values closer to each other are more dissimilar than could be expected for random pair of observations (Legendre 1993). In a system exhibiting no spatial autocorrelation, instead, similar values tend to be sparsely and randomly distributed; a value of an observation at one location cannot be predicted by a value of an observation at another location (Legendre & Legendre 2012). Spatial autocorrelation is especially encountered in studies conducted at small spatial scales, when the data is collected from a limited area (Rushton et al. 2004). This may cause an appearance of a false gradient, which are simply caused by autocorrelated larger-scale spatial structures (Legendre 1993).

Although atypical of spatially structured ecosystems, the independence of observation values is essential in statistical analyses (McGullagh & Nelder 1983, Fortin et al. 1989). Highly positively or negatively autocorrelated data may yield excessively over- or underestimated significance of the model results, respectively, and thus violate the reliability of the model. Hence, testing for independence of the observation values is essential for choosing the valid method for classic statistical analyses (Legendre 1993). Patterns of spatial autocorrelation were evaluated for a set of environmental variables and species richness by Moran's *I* (Moran 1950), an index based on pairwise correlations between observation points inside distinct distance classes. As a sensitive and efficient measure of the degree of spatial autocorrelation in the data, Moran's *I* give as a result correlation coefficient values ranging from -1 to 1. Positive, statistically significant values indicate non-random similarity and clustering of pairs of localities within a given distance class, whereas significant negative values are an indication of dissimilarity between and thus segregation of observation values for a variable (Fortin et al. 1989, Legendre & Legendre 2012).

The use of Moran's *I* involves dividing the geographical gradient into equally wide distance classes. With the studied rock pools, the distance to the sea was considered more important gradient for pool species and communities than the horizontal distance along the shoreline. Since Moran's *I* automatically calculates the coefficients for the longest geographical gradient, the value of each pool Y coordinate was set to zero while keeping the X coordinates unmodified. This ensured that the results were attained for the shorter geographical gradient perpendicular

to the shoreline, described as pool X coordinates. The longest geographical distance from the shore between the studied rock pools was slightly less than 9 m. Thud, the data was divided into five separate distance classes at 2.5 m intervals. The distance classes include pairs of point localities whose coefficients are compared in the analysis. With small datasets sampled at very small spatial scales, small number of distance classes ensures sufficient number of connections between the pools in the analysis. With five distance classes, the minimum number of connections between pairs of sites was 24, which was considered sufficient. Spatial autocorrelation analysis conducts several significance tests simultaneously to all correlation coefficients in each distance class. The number of statistical significance tests affects the significance of spatial autocorrelation. Hence, the p-values of the correlation coefficients were Bonferroni-corrected to consider the number of distance classes in the analysis. The Bonferroni transformation was performed with a formula $\alpha' = \alpha/k$, where k is the number of distance classes and α the significance level of 0.05 (Legendre & Legendre 2012). Every correlation coefficient below $0.05/5 \approx 0.01$ were considered statistically significant.

Spatial correlograms were plotted from the results for visualization of the level of autocorrelation as a function of spatial distance describing spatial dependence between distance classes and correlation coefficients. The shape of the correlogram refers to the spatial pattern of each variable and to the strength of correlation, often revealing either gradients or patch-like structures – the most common patterns of spatial autocorrelation (Fortin et al. 1989, Legendre 1993, Heino et al. 2010). Varying positive and negative statistically significant correlations refer to the patchiness of the values – i.e. small gradients separated by discontinuities. Patchiness is a typical feature of many geographical phenomena over multiple spatial scales (Legendre 1993). Statistically significant, monotonically from positive to negative decreasing correlations are a sign of linearity of the values, while flat correlograms indicate lack of any spatial autocorrelation. Small, statistically non-significant correlations refer to data of completely randomly distributed values. Every correlogram with even a single correlation coefficient below Bonferroni-corrected significance level in a single distance class were considered as statistically significant as whole (Fortin et al. 1989).

6.2.3. Generalized linear models

The most important explanatory variables affecting species richness in each month were examined by *generalized linear models*, or GLMs. GLMs are extensions of classical linear models, which − unlike pure linear models − are also suitable for non-normally distributed

variables often encountered when dealing with ecological data (McCullagh & Nelder 1983). Due to their flexibility, GLMs are especially well suited for and popular in modeling ecological phenomena (Guisan et al. 2002, Rushton et al. 2004). In GLM models, the variation in response variable is explained with a set of multiple potentially predictive independent explanatory variables (Legendre 1993). According to Rushton et al. (2004), GLM consists of three different components: a linear predictor which relates the response variable to the explanatory variables, a link function which relates the linear predictor to the expected value of the response, and an error distribution. The method also allows inclusion of not only first order but polynomial terms as well in the predictor variable set. Since a clear majority of the explanatory variables were non-unimodally distributed, the monthly full GLM models only include second level terms of two clearly unimodal variables; for other variables, only first level terms were used. GLM allows the usage of several different probability distributions to better fit the common non-normal error distribution of observation values (Guisan et al. 2002). The distribution applied to the models is determined by the nature of observation values. Since the response variable of species richness consisted of positive integers without a finite upper limit, the models were based on Poisson error distribution designed for count data. In the nominal Poisson distribution, the positive mean of the observation values is used to determine the error distribution (McCullagh & Nelder 1983).

Selection of explanatory variables to the models was based on the principle of parsimony, a tradeoff between model bias and variance (Johnson & Omland 2004). According to parsimony, the best linear model is the simplest one, in which variability of the response variable is explained with fewest explanatory variables possible. Too many model parameters may result in high variance in parameter estimators and eventually to overfitting of the model with an exceptionally good fit, complicating the identification of irrelevant variables (Rushton et al. 2004). This acquires removal of part of the predictor variables in the model, which lowers the model variance (Guisan et al. 2002). For this reason, explanatory variables were removed from the monthly GLM models by an automatic stepwise method of *MASS* package in R software (Venables & Ripley 2002). The AIC value (*Akaike's information criterion,* Akaike 1973, 1974) considers the number of explanatory variables in the model and is often applied to model selection in ecology (Johnson & Omland 2004). The favor of the AIC value is based on its ability to automatically consider the number of variables in the model, which has proven a convenient way of selecting the best model from a set of alternative models (Rushton et al. 2004). With the backward stepwise method variables were removed from the models one by

one to reduce the AIC value of the models to the lowest level possible. Thus, the GLM model with the lowest AIC value was considered the best, strongly supporting the principle of parsimony.

Explanatory variables of the full monthly GLMs included environmental and spatial variables, excluding monthly multicollinear variables. In May, none of the explanatory variables showed a unimodal relationship with species richness; thus, only the first terms of the variables were included in the model. After the removal of explanatory variables by stepwise backward method, three variables were left to the model: water temperature, pool area and Y coordinates. Of the variables in the full GLM of June, water conductivity had a unimodal relationship with species richness: hence, the second term of this variable was also included in the model. The stepwise backward removal of explanatory variables left three variables to the model: the first and the second terms of water conductivity, together with pool area and X coordinates. In July, pool X coordinates formed a unimodal relationship with species richness, resulting in the inclusion of also the second term of this variable in the full model. Three variables were left to the reduced model: water conductivity and the first and the second term of pool X coordinates.

Critical examination of the produced model involves not only considering the AIC value, but some other measures of the model fit as well (Rushton et al. 2004). The statistical significance of the variables in the reduced models was evaluated with a Pearson chi-square (χ^2) test, which is suitable for Poisson distribution and preferred over other significance tests for its rather direct interpretation (McCullagh & Nelder 1983, Crawley 2007). The explanatory power (D^2) of the models was calculated by reducing residual deviance from null deviance, dividing the result by null deviance, and by multiplying the remainder by 100 (Nakagawa & Schielzeth 2012). Finally, the monthly GLM results were documented in a single table.

6.2.4. Redundancy analysis

Two constrained ordination techniques are usually applied to exploring variables behind variation in ecological data: *canonical correspondence analysis*, or CCA (ter Braak 1986), and *redundancy analysis*, or RDA (van den Wollenberg 1977, Borcard et al. 1992). Traditionally, the former has been thought of better suited for long and unimodal gradients with multiple zeros in the dataset, whilst the latter is better designed to shorter, linear or random gradients with few null observations (ter Braak 1986, 1994, Legendre 1993, ter Braak & Verdonschot 1995). Since the data of this study consists of mostly short, non-unimodal gradients, RDA was favored over

CCA in detecting the most influential explanatory variables for diatom community composition. Despite better fit for short gradients, RDA can be applied to longer gradients as well. The problem of raw species abundancies and consequent null observations can be overcome by Hellinger transformation (Legendre & Gallagher 2001). With Hellinger transformation, the species data is converted to a suitable form for the Euclidean-based RDA to produce more accurate results of the variation explained while preserving the Hellinger distances during the analysis (Legendre et al. 2005, Peres-Neto et al. 2006). RDA is a direct gradient analysis, in which the relative importance of explanatory variables for the response variable is determined by relating species observations directly to a set of explanatory variables (ter Braak 1986, 1994). The analysis is asymmetric, meaning that the data matrices used in the analysis differ in their roles. The response variables – i.e. the species compositional data – constitute the first matrix, while the other is formed by explanatory variables used to explain the variation in the species data matrix. As a result, RDA produces ordinations of community composition matrix constrained by the explanatory matrix (Legendre et al. 2011).

The result of RDA is an ordination diagram, or a biplot of two canonical, constrained ordination axes, formed by linear combinations of the explanatory variables (Legendre et al. 2011). Each axis has an eigenvalue, which is a measure of the quality of the resulting ordination and the strength of the relationship between species and environment (ter Braak 1994). The ordination axes divide the biplot to separate sections that differ by their species composition and habitat characteristics. The relative importance of the quantitative explanatory variables to the response variable is illustrated by arrow-shaped vectors of differing lengths: the longer the arrow, the more important the given variable to the species composition (ter Braak 1986, 1987). The ordination direction of the arrows in relation to the ordination axis is either positive or negative. Lengthening of the arrow indicates increasing rate of change in the values of the explanatory variable toward the positive end of the axis (ter Braak & Verdonschot 1995). In addition to the relative importance of different explanatory variables, the biplot reveals point location of individual species, habitat sites and qualitative explanatory variables in relation to the ordination axes and quantitative explanatory variables. These represents the dominant compositional patterns in the studied community (ter Braak 1986, 1987, ter Braak & Verdonschot 1995). Species and sites – i.e. rock pools – located closer to each other indicate more similar environmental and spatial conditions and distribution along these requirements, whilst longer distances between the two are an indication of dissimilar environments or diverging species preferences. Species located in the center of the plot near the origin of the

axes are either not affected by the set of explanatory variables or have their actual optima in the origin (ter Braak 1987, ter Braak 1994, ter Braak & Verdonschot 1995).

RDA was conducted separately to each monthly dataset; in addition, a fourth RDA was done for the combined dataset consisting of all the monthly species observations and variable records. Explanatory variables in the analyses included all non-multicollinear environmental and spatial variables. The number of explanatory variables for each month was considered reasonably low, with no need for further reducing the number of variables for the analysis (Legendre et al. 2011). All the models included the same variable set with two exceptions. In June, pool Y coordinates were excluded from the variables, and for the combined RDA of all monthly records, a three-category nominal variable 'month' was added to describe temporal variability in the monthly records from May to July. The statistical significance of the explanatory variables, ordination axes and the model as whole were assessed by the F-test for each RDA. The significance of individual axes determines the extent to which the axes represent variation in the community that can be separated from purely random (Legendre et al. 2011).

6.2.5. Variation partitioning

Variation partitioning (Borcard et al. 1992) is the most widely applied statistical method in ecology to study the relative influence of environment and space for community composition. The method partitions variation in species abundance data to different components according to both environmental and spatial variables (Logue et al. 2011). Variation partitioning was conducted to each monthly species dataset to reveal separate and shared proportional effects of environmental and spatial variables on diatom community composition. Spatial variables were pool X and Y coordinates; environmental variables consisted of water physiochemical characteristics and pool morphometrics.

The results of the variance partitioning share the explained variation in species community composition to fractions explained by pure spatial ([S]) and pure environmental factors ([E]), as well as by their joint effect expressed as spatially structured environmental variation ([E∩S]) (Legendre et al. 2005). If the fraction explained by pure environmental factors is the largest, species community is mainly regulated by changing environment. This means that spatial factors alone – or as a part of shared effects with environmental factors – do not affect the community to a great extent, and species in the community are sorted to different habitats by their environmental preferences. If the fraction explained by pure spatial factors is the largest, on the other hand, species communities are mainly regulated by geographical distance between

the habitats. In this case, the measured environmental conditions are virtually meaningless for the species to exist; instead, species occurrence is regulated by their dispersal ability between habitats in different locations. If the shared fraction of the explained variance in species composition is the largest of the three, both environment and space are important for regulating species occurrence.

The statistical significance of two of the model components – i.e. fractions explained by pure environmental and pure spatial factors – was assessed. This was done by redundancy analysis for both sets of explanatory variables – the spatial and the environmental ones. RDA was run separately for both variable sets and the significance of the resulting model tested by the F-test (Legendre 1993). If both components are to be statistically significant – and especially if the variation explained by both is larger than the shared effect of these variable groups on the species community – these two groups of variables can be considered to affect the species composition. This implies that though the other variable group – i.e. environmental or spatial variables – may be more significant or explain more of the variation, the species composition is to a certain extent regulated by both the environment and space. The significance of pure effects of these two components also confirms that neither of the processes simply covary with each other, but truly have impacts of their own for the response variable (Soininen & Weckström 2009).

6.2.6. Mantel and partial Mantel tests

Variance in community composition does not translate directly to variance in community similarity. Unlike canonical analyses, which partition the variation in species abundance data and is an appropriate method for testing variation in community composition based on raw species abundancies, a separate distance-based statistical method is needed for partitioning the variation in pairwise dissimilarities among study sites (Legendre et al. 2005). The change in community similarity along environmental and geographical distance was evaluated with *Mantel test* (Mantel 1967). Mantel test is the most widely used method for determining relationship between two different distance matrices (Legendre 2000). The test reveals the causes of this variation along a distance gradient for transferred, distance-based species matrix (Legendre et al. 2005). The *similarity* is calculated by comparing two observations from pairs of sites in different locations. The similarity between samples can be modeled as a function of inter-sample distance by linear regression, producing a direct estimate of the rate of distance decay (Nekola & White 1999). By linear multiple regression, Mantel test analyzes the

community composition distance matrix against environmental and spatial distance matrices (Legendre 2000). The two latter are explanatory matrices which are used to explain variation in the response matrix of community composition. The result thus reflects the change in the resemblance of community structure along geographical or environmental gradient (De Cáceres et al. 2013). The ability of Mantel test to explain the variation is expressed as correlation coefficient (R^2), which simply indicates the fit of the data to each monthly model (Legendre et al. 2005).

Mantel tests were performed for each monthly species dataset to reveal the effects of spatial and environmental variables on the change of community composition along geographical distance gradient. The environmental data comprised monthly water physiochemical characteristics and pool morphometrics; spatial variables consisted simply of pool X and Y coordinates. Three distance matrices were created from the data: a biotic matrix of species abundancies, an environmental matrix of water physiochemical characteristics and pool morphometrics and a spatial matrix of pool X and Y coordinates. A Bray-Curtis dissimilarity index (Bray & Curtis 1957) was used to describe the change of community similarity along the geographical distance. The index gains values from 0 to 1, low values indicating similarity of species composition between sites. As a sensitive measure of similarity based on species abundancies, it is among the most reliable and widely used similarity indices in ecology (Bloom 1981, Clarke 1993, Nekola & White 1999). Significance of the Mantel correlations were computed with 9999 permutations. The results of monthly Mantel tests were plotted and documented to a table. The plots reveal the degree of distance decay in the community. A rapid change in species composition with spatial or environmental distance usually implies high among-pool beta diversity. This is seen as a steep curve on the plot describing the relationship between distance and species composition. Conversely, gentler curve indicates slower change of species composition with increasing distance. Simultaneously with Mantel test for spatial variables, halving distance for species composition was calculated. Halving distance can be calculated for any regression line, thus enabling comparisons of distance decay between different communities (Soininen et al. 2007). The halving distance was calculated for the regression line with a formula $(\beta-\alpha)/2\beta$, where β is the regression coefficient and α the interception of y axis and the regression line. From the regression line, initial similarity was determined at 1 m distance (Soininen et al. 2007).

Environmental conditions tend to change along increasing geographical distance, which may hinder the single effects of these variables on the species composition. Even a major change in species composition along the spatial gradient may not thus describe the pure change of species composition by increasing distance, but of the change of species by also some changing environmental factors. For this reason, *partial Mantel test* (Smouse et al. 1986) was conducted for each monthly species data set. Partial Mantel test is a first-order partial correlation analysis widely used in ecological studies. As an extension of Mantel test, the analysis involves three distance matrices: one for species abundancies and two others for environmental and spatial variables. The difference to the traditional Mantel test is that while Mantel test compares two matrices at a time, partial Mantel test compares all three distance matrices simultaneously (Legendre 2000). The advantage of partial Mantel test is thus that it enables controlling for the third matrix while investigating the relationship between the two other matrices (Vanschoenwinkel et al. 2007, Legendre & Legendre 2012). The explanatory variable sets were the same as in Mantel test. The test divides the variation in distances among sites explained by pure environmental ([E]|[S]) and pure spatial ([S]|[E]) variables, excluding the effects of the other variable set. Significance of the correlations were computed with 9999 permutations. The results of partial Mantel tests were documented in the same table with Mantel test results.

7. RESULTS

7.1. Variation in explanatory variables and species richness

7.1.1. Environmental and spatial variables

High environmental heterogeneity among the rock pools was detected throughout the study. Most of this variability was due to differences in water physiochemical characteristics, with a lesser contribution of pool morphology. In general, the studied pools were small and shallow – a pattern that remained constant throughout the study period (Fig. 1). Temporal variation in pool size was likewise minor (Table 1). For example, the maximum depth of the pools was less than 0.5 m throughout the study period, while the mean area stayed below 5 m² in each month. However, three of the pools stood distinctly out as deeper and larger by both area and volume in two of the three study months. Especially the area of two of the pools increased considerably by several square meters from May to July.

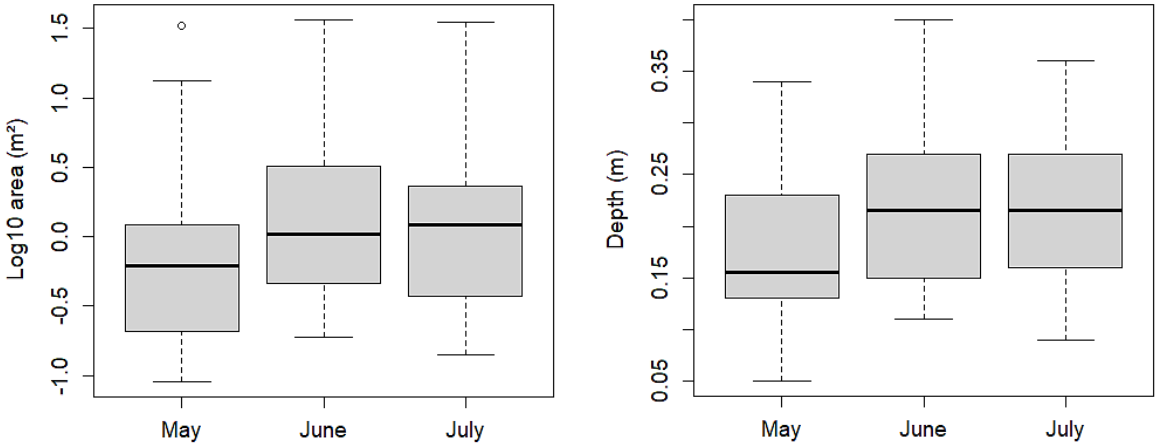


Figure 1. Boxplots of pool morphological characteristics between sampling months. Shown are values for monthly median (the black line), lower and upper quartiles (the box vertical limits), lower and upper 95-percentiles (the whiskers) and outliers falling 1.5-fold outside the quartiles (white dots) for pool depth and area. Pool area is calculated from the monthly measures of pool maximum length and width. Values of pool area are $\log_{10}$-transformed.

Considerable spatial and temporal variation was detected among water physiochemical characteristics of the studied pools (Table 1, Fig. 2-3). The nutrient concentrations were high throughout the study period. The spatial differences in total phosphorus concentrations were by far greatest in May and smallest in June. The spatial variability in total nitrogen concentrations decreased from May to July with rather steadily declining minimum and maximum values but stayed high throughout the study period. However, significant temporal variation in total P was detected among the pools, with local concentrations as high as 1,448.3 µg/L in May. The mean total N concentration decreased from May to June but increased slightly again in July.

Water conductivity varied greatly among the studied pools every month. The spatial differences in among-pool measures were most evident in May. In general, extremely high maximum conductivities were recorded among the pools throughout the study period, with a maximum conductivity of 16,741.0 µS/cm measured in May. In June, the conductivity gradient was the lowest, but the mean conductivity was the highest. The lowest mean conductivity was measured in July, but few extremely high measured conductivities increased the gradient of July beyond that of June's. Conversely, the high mean conductivity in June is a result of spatially uniform distribution of conductivity measures in that month. The results thus clearly reflect spatial differences in monthly measures, especially when concerning the last two months.

Table 1. Monthly statistics for the environmental and spatial variables and the response variable. Spatial variables are only shown for May. The X and Y coordinates are based on estimated measures from a grid map drawn of the study area. The pool isolation is the average distance from a pool to 5 geographically closest pools.

Month	Variable	Min	Max	Mean	Median	Std. dev.
May	Environmental variables					
	Total phosphorus (µg/L)	49.1	1448.3	378.4	270.2	348.2
	Total nitrogen (µg/L)	737.5	8750.0	3495.1	2643.8	2265.2
	pH	8.1	9.9	9.2	9.3	0.5
	Conductivity (µS/cm)	134	16741	3964	2014	4421
	Temperature (°C)	18.5	26.2	21.3	21.3	1.7
	Depth (m)	0.05	0.34	0.17	0.16	0.08
	Area (m²)	0.09	33.12	2.71	0.62	6.52
	Spatial variables					
	X coordinate (m)	1.6	11.4	6.6	6.9	2.7
	Y coordinate (m)	1.4	106.2	50.5	49.6	28.3
	Isolation (m)	2.7	18.9	7.7	7.1	4.3
	Response variable					
	Species richness	21	50	34	36	7
June	Environmental variables					
	Total phosphorus (µg/L)	17.3	363.1	82.1	65.3	71.4
	Total nitrogen (µg/L)	500.0	7425.0	1997.0	1372.0	1689.3
	pH	7.2	10.0	8.7	8.8	0.8
	Conductivity (µS/cm)	62	10754	4438	3868	4005
	Temperature (°C)	14.8	21.3	18.7	18.9	1.6
	Depth (m)	0.11	0.40	0.23	0.22	0.08
	Area (m²)	0.19	36.72	4.01	1.04	8.10
	Response variable					
	Species richness	18	52	34	33	9
July	Environmental variables					
	Total phosphorus (µg/L)	22.2	724.8	181.5	139.7	165.1
	Total nitrogen (µg/L)	400.0	6100.0	2201.0	1700.0	1427.2
	pH	6.7	9.5	7.9	7.9	0.6
	Conductivity (µS/cm)	38	14383	3902	943	4818
	Temperature (°C)	15.8	20.7	19.2	19.6	1.2
	Depth (m)	0.09	0.36	0.21	0.22	0.1
	Area (m²)	0.14	34.98	4.72	1.24	9.0
	Response variable					
	Species richness	5	46	27	28	8

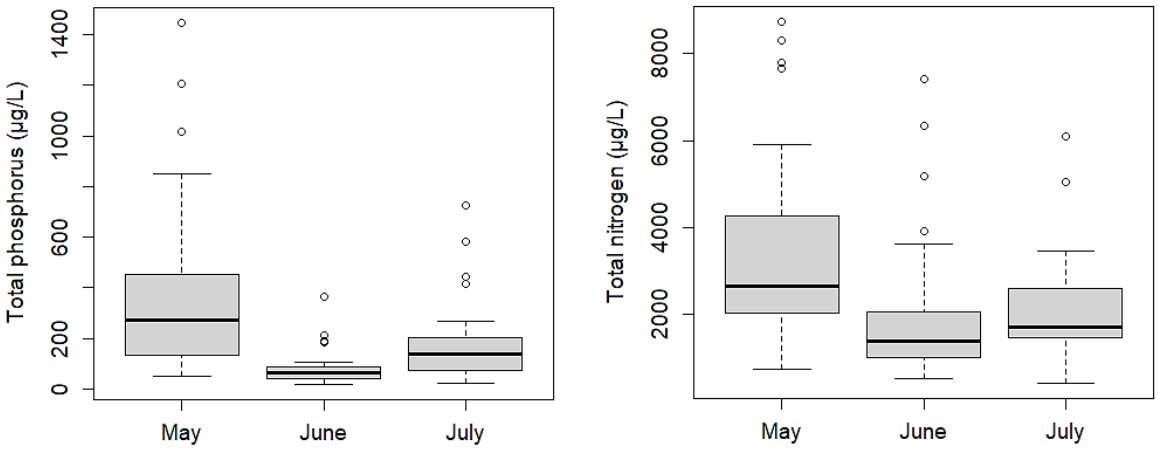


Figure 2. Boxplots of total phosphorus and total nitrogen for the studied pools between sampling months. For figure details, see Fig. 1.

Spatial differences in water pH were lowest in May and highest in June. On average, all studied pools were alkaline from May to July, with mean pH values exceeding 7.5 every month. Part of the pools were clearly alkaline throughout the study period with water pH higher than 9.0. In July, however, two of the pools were near acidic with pH values slightly below 7.0. A clear temporal gradient was detected in water pH during the summer, the pool water being most alkaline in May and closest to neutral in July. By temperature, the pool water was warm in May and cooler in June and July, varying by several degrees between the months. The coldest pool water was measured in June. In July, the temperatures slightly increased from June, but stayed moderate in comparison with the warmest month, that of May. In general, spatial variability in water temperatures was rather low, with moderately decreasing spatial temperature gradients from May onwards.

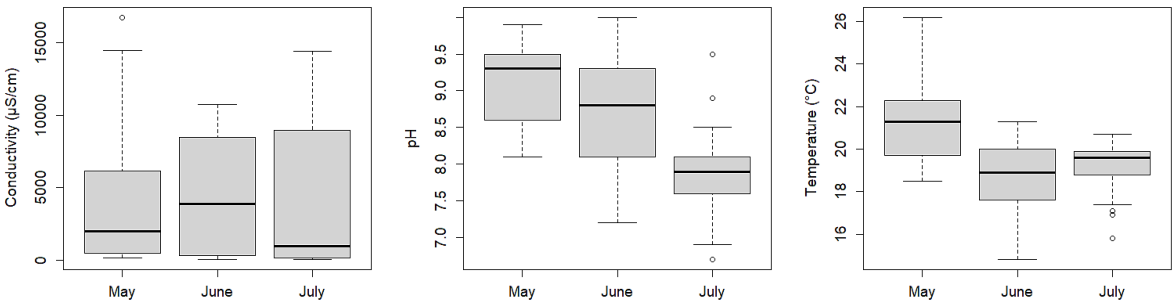


Figure 3. Boxplots of water physiochemical characteristics for conductivity, pH and temperature for the studied pools between sampling months. For figure details, see Fig. 1.

Due to the small study scale of only 1,200 m², spatial gradients were generally short by nature (Table 1). The horizontal location of the studied pools parallel to the shoreline – i.e. pool Y coordinates – formed a gradient of c. 105 m. Most of the pools were located beyond 50 m from the southern edge of the study area. The X coordinates reflected the pool distance to the sea, being based on the perpendicular distance of the pools from the shoreline. In July, the X coordinate of pool 26 differed from the distance to the sea by 2.2 m. This was due to the overflowing of the pool by rising sea level, which ultimately merged the pool water directly with the adjacent sea. Though the distance to the sea thus decreased from 2.2 m to practically zero in July, the geographical location of the pool in respect to the other pools was considered constant, keeping the X coordinate of the pool same every month. The distance to the Baltic Sea formed a spatial gradient of c. 9 m. On average, the pools were located 6.6 m from the sea, whilst majority of the pools were located beyond 5 m from the seashore. The isolation of the pools, i.e. the mean distance to five geographically closest pools of a given pool, ranged between 2.7 and 18.9 m. Majority of the pools were not located farther than 10 m from the five closest pools.

Several correlations between explanatory variables were detected in each month (Table 2, Appendix 1). Pool morphometrics were highly correlated with each other every month, several of the correlations exceeding the limit of multicollinearity. The strongest single correlation of all explanatory variables in each month was detected between pool area and volume, the two variables being highly multicollinear in each month. Other strong correlations were detected among water nutrient concentrations. Total phosphorus and total nitrogen were highly correlated with each other every month, being strongly multicollinear in May and July. Occasionally, both total P and total N correlated negatively with pool morphometrics, whilst total N was partly related to pool water chemistry and pool location. Water temperature and conductivity were mostly related to pool location, the latter being multicollinear with pool Y coordinates in June. The correlations between species richness and the explanatory variables were generally weak every month. In May and June, no statistically significant correlations were detected. In July, species richness correlated negatively with pool morphometrics. Negative unimodal relationships between species richness and the explanatory variables were detected in June with water conductivity and in July with the X coordinates.

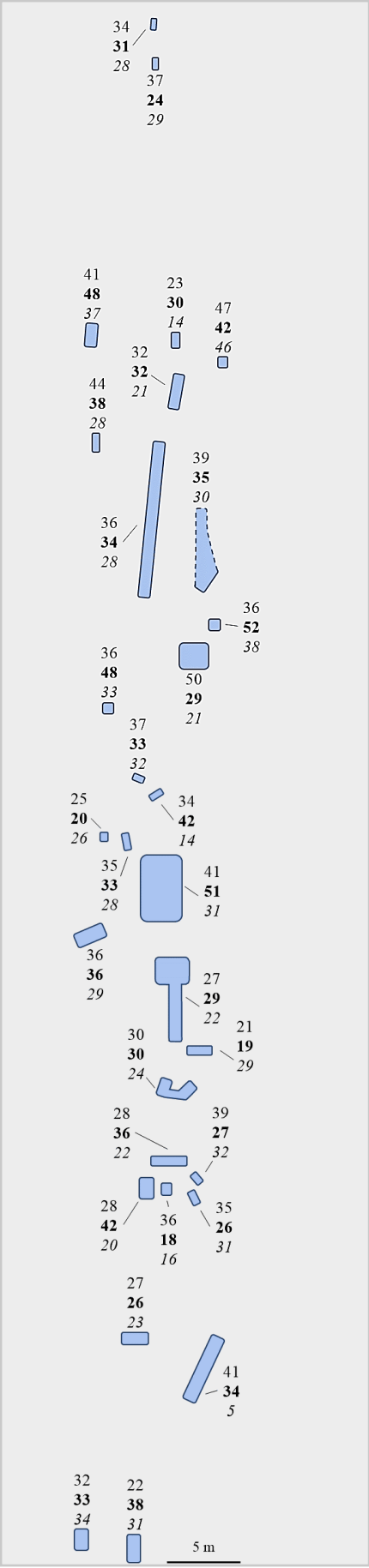
Table 2. The Spearman rank correlation coefficients and the statistical significance of correlations for the response variable and environmental and spatial variables in May. Shown are three significance levels according to the p-value of each pairwise correlation: - non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Bolded correlation coefficients are statistically significant ($p < 0.05$). For correlations between variables in June and July, see Appendix 1.

	Total P	Total N	pH	Conductivity	Temperature	Lenght	Width	Depth	Area	Volume	X coordinate	Y coordinate	Isolation	Species Richness
Total P		**0.73**	0.14	-0.20	0.06	**-0.37**	-0.35	**-0.65**	**-0.39**	**-0.49**	0.07	0.09	0.13	-0.13
Total N	***		0.06	-0.15	0.14	**-0.44**	-0.33	**-0.52**	**-0.43**	**-0.47**	0.12	0.09	-0.28	-0.21
pH	-	-		0.17	0.12	0.05	0.08	0.13	0.13	0.19	-0.14	-0.12	0.16	-0.05
Conductivity	-	-	-		-0.07	0.19	**0.44**	0.04	**0.37**	0.35	**-0.45**	**-0.62**	-0.12	-0.35
Temperature	-	-	-	-		0.03	-0.01	-0.07	-0.01	-0.03	-0.35	**0.43**	-0.07	-0.06
Length	*	*	-	-	-		**0.67**	**0.54**	**0.89**	**0.85**	-0.02	-0.18	0.02	0.28
Width	-	-	-	*	-	***		**0.58**	**0.91**	**0.89**	-0.01	**-0.43**	-0.23	-0.03
Depth	***	**	-	-	-	**	***		**0.62**	**0.78**	0.21	-0.11	-0.26	0.17
Area	*	*	-	*	-	***	***	***		**0.96**	0.05	**-0.37**	-0.10	0.14
Volume	**	**	-	-	-	***	***	***	***		0.06	-0.35	-0.16	0.13
X coord.	-	-	-	*	-	-	-	-	-	-		0.04	-0.25	0.15
Y coord.	-	-	-	***	*	-	*	-	*	-	-		0.25	0.36
Isolation	-	-	-	-	-	-	-	-	-	-	-	-		0.15
Sp. Richness	-	-	-	-	-	-	-	-	-	-	-	-	-	

Spatial autocorrelation – measured along the diagonal gradient from the coastline toward inland – between the variables was weak in general (Appendix 2). In May, none of the variables were spatially autocorrelated in any distance class. In June, the correlation between water depth and spatial coordinates was significant in the first distance class according to the Bonferroni-corrected level of significance ($p = 0.05/5 = 0.01$); this correlogram was considered statistically significant as whole. In July, significant autocorrelation was detected for total phosphorus, total nitrogen, water conductivity and temperature in the first distance class. For conductivity, spatial autocorrelation was also significant in the second distance class. These four correlograms were likewise considered globally statistically significant. For all other explanatory variables and for species richness, no significant spatial autocorrelation was detected in any of the months.

7.1.2. Species richness and abundance

A total of 179 species were detected during the study period from May to July. In May, the number of detected species was 127. In June, the corresponding figure was 140, and in July 122. Temporal variation in species richness was locally high (Fig. 4). The species richness of a pool varied from a minimum of five recorded species in July to a maximum of 52 species in



June (Table 1). Spatial variation increased from May to July, the latter month deviating from the others by its lower total and higher range of species richness. The number of species includes all the detected species identified to both species and genus level.

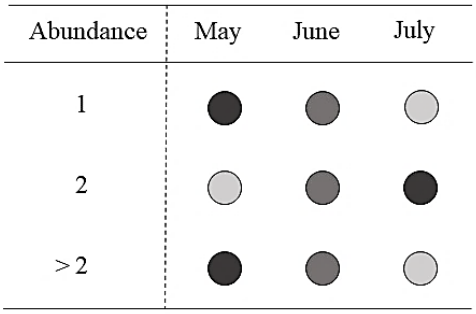
Majority of the species were rare, occurring as few cells only. One third (33%) of all detected 179 species were present in one single month only. Of these species, 31% were detected in May alone, 42% in June, and 27% in July. Majority of these species, i.e. 62%, occurred as a one cell. Thus, one fifth (20%) of all the species detected every month were extremely rare, only occurring as a one single individual in one single pool in only one of the months. Majority of these species were present in May, the proportion decreasing toward July (Table 4). Same trend was evident for species occurring in more than two cells, whilst the opposite was detected for species occurring as two cells only, with the highest abundancies recorded in July and the lowest in May. Of all 179 species, 17% were detected in two of the study months. Nearly half (47%) of these species were absent in July. Half (50%) of all detected 179 species occurred every month. Approximately one quarter of these species were extremely abundant, occurring as thousands of cells at least in two months. An ultimate example of this pattern is species *Tabularia fasciculata*, which was present every month in nearly every pool (Table 3). In May and July, a few species frequently occurred in every pool. In July, none of the species occurred in all pools. Instead, the commonest species *Rhoicosphenia abbreviata* occurred in 97% of the pools.

Figure 4. Monthly species richness of the studied rock pools. The three figures above each pool refer to the number of species in each month: normal text = May, **bolded** = June, *italic* = July. For pool numbering and map details, see Fig. 2 in chapter 5.

Table 3. The most frequently occurring diatom species present in the studied rock pools from May to July. Shown are the 11 commonest species in each month ranked by the percentage of occupied pools by each of the species and the mean and the range of per-pool species abundancies (%).

Month	Species	Occupancy (%)	Abundance (range) (%)
May	*Diatoma moniliformis* (Kützing) D.M.Williams	100	5.2 (0.3-21.8)
	Navicula perminuta Grunow	100	9.2 (1.7-29.9)
	Tabularia fasciculata (C.Agardh) D.M.Williams & Round	100	2.8 (0.3-9.7)
	Achnathidium minutissimum (Kützing) Czarnecki	97	12.1 (0.2-66.4)
	Navicula gregaria Donkin	97	2.7 (0.2-10.4)
	Navicula lanceolata Ehrenberg	97	1.9 (0.2-7.4)
	Navicula recens (Lange-Bertalot) Lange-Bertalot	97	0.8 (0.2-3.9)
	Nitzschia frustulum (Kützing) Grunow	97	5.4 (0.2-22.0)
	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	97	1.4 (0.2-4.6)
	Nitzschia inconspicua Grunow	90	4.0 (0.2-21.3)
	Surirella brebissonii Krammer & Lange-Bertalot	90	5.7 (0.3-50.6)
June	*Diatoma moniliformis* (Kützing) D.M.Williams	100	7.0 (0.4-41.4)
	Navicula gregaria Donkin	100	2.2 (0.2-11.3)
	Nitzschia frustulum (Kützing) Grunow	100	4.0 (0.3-18.8)
	Tabularia fasciculata (C.Agardh) D.M.Williams & Round	100	4.1 (0.2-25.7)
	Navicula perminuta Grunow	97	5.2 (0.3-17.6)
	Nitzschia microcephala Grunow	97	24.5 (0.2-83.2)
	Achnathidium minutissimum (Kützing) Czarnecki	90	10.2 (0.2-43.4)
	Planothidium delicatulum (Kützing) Round & Bukhtiyarova	87	0.9 (0.2-4.0)
	Nitzschia inconspicua Grunow	83	1.5 (0.2-6.9)
	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	83	0.9 (0.2-3.2)
	Nitzschia pusilla Grunow	80	5.1 (0.2-21.7)
July	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot	97	2.3 (0.2-16.3)
	Cocconeis placentula Ehrenberg	93	1.5 (0.2-10.2)
	Tabularia fasciculata (C.Agardh) D.M.Williams & Round	93	2.4 (0.2-13.7)
	Navicula gregaria Donkin	87	1.4 (0.2-10.4)
	Navicula perminuta Grunow	87	3.1 (0.2-16.1)
	Diatoma moniliformis (Kützing) D.M.Williams	80	3.4 (0.2-21.5)
	Nitzschia microcephala Grunow	80	32.5 (0.2-90.0)
	Achnathidium minutissimum (Kützing) Czarnecki	77	11.5 (0.2-55.4)
	Nitzschia frustulum (Kützing) Grunow	77	2.5 (0.2-11.6)
	Nitzschia pusilla Grunow	73	5.1 (0.2-27.0)
	Surirella brebissonii Krammer & Lange-Bertalot	70	3.2 (0.2-21.9)

Table 4. Temporal variation of species rarity between the sampling months. The abundance refers to diatom cells detected as only 1, only 2 or more than 2 cells. The dots represent the ranked rarity of each abundance class from the most abundant (dark grey) to the least abundant (light grey) between the three months. E.g., the abundance of species occurring as one cell only was highest in May and lowest in July.



A total of 62 different diatom genera were detected during the study. Of these genera, majority (73%) was represented by a maximum of two species, and 55% by only one species. Two subspecies were identified of one *Gomphonema exiguum* species, *G. exiguum var. exiguum* and *G. exiguum var. minutissimum*. Nine species were identified to a genus level, of which four belonged to the *Navicula* genus, two to *Achnanthes* genus, and the rest to *Nitzschia*, *Pinnularia*, and *Thalassiosira* genus, one species of each. By species richness, most diverse genera were *Nitzschia* (29 spp.) and *Navicula* (26 spp.). Other diverse genera were *Gomphonema* (10 spp.), *Eunotia* and *Pinnularia* (both 8 spp.). Approximately 38% and 35% of the species in *Pinnularia* and *Nitzschia* genera, respectively, were detected in one month only. Of the *Navicula* and *Eunotia* genera, roughly a quarter of the species were detected in one month only. The species of the genus *Gomphonema* occurred throughout the study period. In May and June, the most abundant species was *Nitzschia microcephala*, which comprised 11.9% and 24.0% of all detected individuals in these months, respectively. In July, the most abundant species was *Cyclotella meneghiniana* with a 10.3% share of all detected species. *Achnathidium minutissimum* was also amongst the most abundant species every month, ranked on the second place from May to June and third on July. The most frequently occurring species were usually the most abundant ones, but not all the abundant species were general. For example, of the individuals of *Nitzschia palea* and *Gomphonema gracile*, the fourth and sixth abundant species in July, up to 69% and 74% occurred in one single pool only, with occupancies of only 53% and 27%, respectively.

7.2. Factors affecting species richness

Few statistically significant explanatory variables were left in the reduced monthly GLMs (Table 5). According to the chi-square test, pool Y coordinates were the only significant explanatory variable in the May's GLM. The AIC value of the model was 201.1, the model explaining 25.7% of the variation in species richness. Of the explanatory variables of June's GLM, only the second term of conductivity was statistically weakly significant. The first term of conductivity was negative and the second term positive, indicating a U-shaped relationship

with species richness. The AIC value of the model was 226.2, and the explanatory power 17.1%. Of the variables of July's GLM, only the second term of pool X coordinates was statistically significant. Also with this variable, the first term was negative and the second positive, indicating a U-shaped relationship with species richness. The July's GLM had an AIC value of 219.4 and an explanatory power of 26.7%.

Table 5. Results of the generalized linear models for each of the study months. Shown are the explanatory variables and their first and second terms left in each model after a backward stepwise removal of the variables from the full monthly models. Shown are also the AIC value and the proportion of explained variance (D^2) of species richness of each model. Significance of the variables is based on the p-value of Chi-square test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Month	Variable	Estimate	Std. Error	z value	Pr(>Chi)	Sig.	AIC	D^2
May	Temperature	-2.881E-02	2.011E-02	-1.433	0.57198		201.1	25.7
	Area	8.173E-03	4.411E-03	1.853	0.07082			
	Y coordinate	3.301E-03	1.188E-03	2.778	0.00581	**		
June	Conductivity	-8.468E-05	3.652E-05	-2.319	0.41373		226.2	17.1
	Conductivity^2	8.195E-09	3.697E-09	2.216	0.02534	*		
	Area	7.636E-03	3.746E-03	2.039	0.05190			
	X coordinate	-1.873E-02	1.275E-02	-1.468	0.14244			
July	Conductivity	-2.291E-05	1.134E-05	-2.021	0.92750		219.4	26.7
	X coordinate	-3.060E-01	6.459E-02	-4.737	0.17900			
	X coordinate^2	2.145E-02	4.721E-03	4.542	0.00001	***		

7.3. Factors affecting community composition

The RDA model of May explained 22.0% of the variation in species community composition. The only statistically significant explanatory variables were water conductivity and pool X coordinates, explaining 6.6% and 3.4% of the variation in community composition, respectively (Table 6). The two first ordination axes explained 19.2% and 9.3% of the variation in community composition. Water conductivity had the strongest correlation with the species matrix along the first ordination axis, while pool X coordinates yielded the highest correlation coefficient on the second axis. The RDA model of June explained 23.1% of the variation in species community. Water conductivity was the most important variable, explaining 9.1% of the variation. Other significant variables were pool X coordinates and water pH. The first and the second ordination axes explained 24.4% and 8.1% of the variation in community composition, respectively. Water conductivity had the strongest correlation with the species matrix along the first ordination axis and pool X coordinates on the second axis. In July, the RDA model explained 30.5% of the variation in community composition. Water conductivity was yet again the most important variable affecting the species composition, explaining 10.2% of the variation in the community. Other significant factors were water temperature and total

phosphorus concentration. The first and the second ordination axes explained 22.4% and 13.2% of the variation in community composition. Of the explanatory variables on the first and the second axes, water conductivity and temperature had the strongest correlation with the species matrix, respectively.

The combined RDA model explained 20.9% of the variation in species community composition. Several variables were highly significant, of which water conductivity was the most important, accounting for 7.6% of the explained variation. The first and the second ordination axes explained 18.2% and 7.5% of the variation in community composition. Water conductivity had the highest correlation coefficient on the first ordination axis, while pool X coordinates had the strongest correlation with the species matrix on the second axis. Of all four RDA models, the model combining all the monthly records explained the lowest share of the variation in community composition, but the significance of single explanatory variables was the highest. For each model except that of July's RDA, the residual variation was consistently higher than the explained variation of the community composition. All the four RDA models were statistically highly significant according to the F-test.

Table 6. The results of the monthly and combined redundancy analyses. Shown are the variance explained by statistically significant explanatory variables in each month, together with their correlation with species matrix along the two first ordination axes. The significance of the variables is based on the p-value of F-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Month	Variable	Variance	RDA 1	RDA 2	Pr(>F)	Sig.
May	Conductivity	0.06603	-0.9148	0.1351	0.001	***
	X coordinate	0.03403	0.3257	-0.8258	0.003	**
June	pH	0.03191	-0.4523	0.0470	0.012	*
	Conductivity	0.09100	-0.9489	-0.1914	0.001	***
	X coordinate	0.03606	0.3091	0.8802	0.014	*
July	tot P	0.03683	0.0679	0.5697	0.019	*
	Conductivity	0.10189	-0.7666	-0.5748	0.001	***
	Temperature	0.03790	0.0666	0.8112	0.013	*
	Isolation	0.03715	0.4444	-0.6187	0.029	*
All	pH	0.01657	-0.1464	-0.4626	0.001	***
	Conductivity	0.07562	-0.8676	-0.3254	0.001	***
	Depth	0.01248	-0.0329	0.0747	0.003	**
	X coordinate	0.02565	0.3395	0.6630	0.001	***
	Y coordinate	0.01387	0.7699	-0.0442	0.005	**
	Isolation	0.02189	0.4437	-0.2560	0.001	***
	Month	0.02245	-0.1119	0.6405	0.002	**

The first and the second ordination axes divided the pools to monthly differing groups by pool location and water physiochemistry (Fig. 5). In May, the pools were nearly uniformly scattered

along the axes. The clearest division of the pools was along the first ordination axis, separating larger pools with alkaline, cooler, less phosphorus-rich but more conductive water near the seashore from smaller pools located farther from the seashore with less alkaline, phosphorus-rich and warm water with lower conductivities. On the biplot of June's model, three pool clusters are evident. On the positive end of the first ordination axis, a cluster of isolated, large and less alkaline pools are separated from a cluster of smaller, less isolated pools with phosphorus-rich and alkaline water. The third group of pools is located on the upper right quarter of the biplot. These pools are deep with warm and less conductive water located farther from the seashore. In July, the aggregation of pools in distinct clusters differing by spatial and environmental characters was most evident. A total of three separate pool categories can be detected from the biplot, two of them being located on the upper half of the plot near the positive end of the second axis. On the right side of the axis, there is a group of deep pools with less conductive and partly warm and phosphorus-rich water lying farther from the sea near the northern corner of the study area. These pools can be separated from the less isolated pools on the left side of the axis. The third pool cluster is found from the negative end of the second ordination axis on the lower part of the biplot. These pools are small, isolated and alkaline with lower phosphorus concentration and a longer distance to the sea.

From the biplot combining all the study records, monthly varying aggregations of the pools along the ordination axes and explanatory variables can be observed (Fig. 5). In May, the pools are scattered rather uniformly on the biplot, being slightly emphasized on the lower side of the plot. The community is mainly regulated by water pH, temperature, and pool isolation. In June, the pools are most evenly scattered on the plot. Several factors affect the species composition, pool X coordinates, isolation, water pH and conductivity being the most important ones. In July, the pools are nearly exclusively located on the upper half of the biplot. Some of the most important factors in this month are pool X coordinates, isolation, water pH and conductivity.

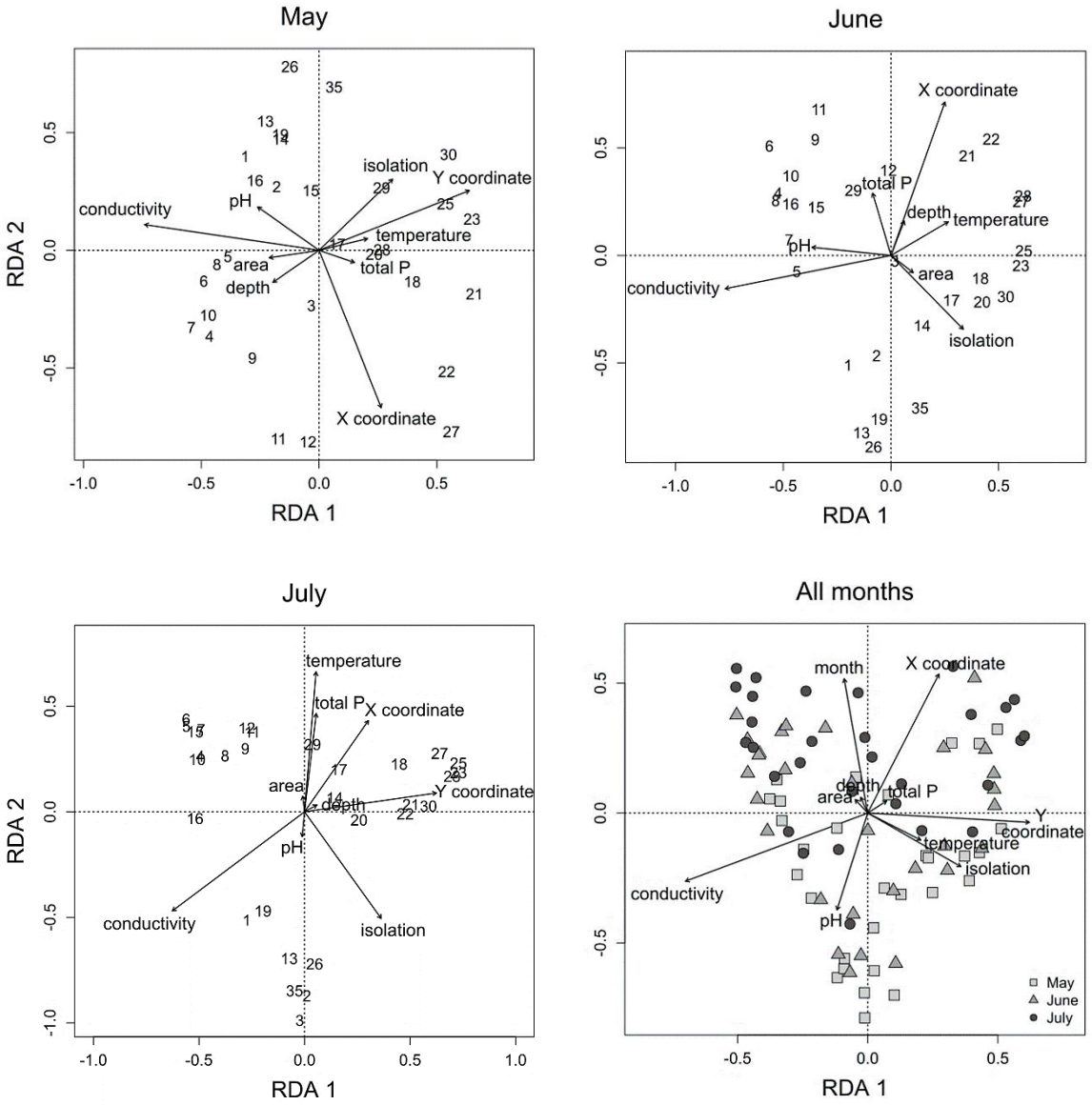


Figure 5. The results for the three monthly redundancy analyses and the one with combined monthly records. Shown are the location of the studied rock pools (numbered from May to July, symbolled in the combined model) in relation to the ordination axes and the explanatory variables.

Variation partitioning divided the variation in species community composition to three distinct fractions explained by pure environmental ([E]), pure spatial ([S]) and jointly by both spatial and environmental factors ([E∩S]). The joint effect of environmental and spatial factors consistently explained the largest share of monthly variation in species community composition (Fig. 6). The variation explained by pure environmental and pure spatial factors was nearly equally low in each month. In May, pure spatial variables accounted for a slightly higher proportion of the explained variation than pure environmental variables, whilst in June and July pure environmental variables explained more of the variation in species composition than pure

spatial ones. The residual variation was nearly equal in each month, leaving more than 75% of the monthly variation in community composition unexplained. Based on the F-test of redundancy analysis, the fractions explained by pure environmental ([E]) and pure spatial components ([S]) were statistically significant in each month.

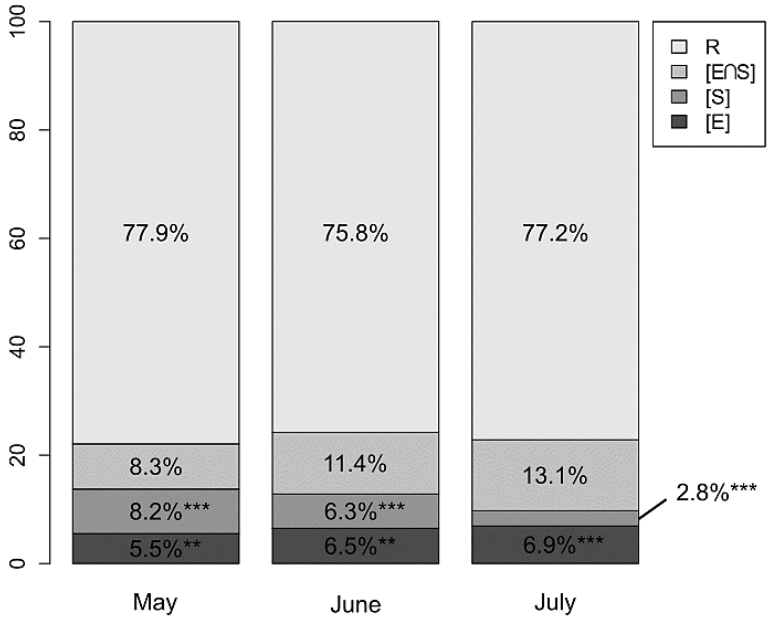


Figure 6. The results for variation partitioning for each of the study months. Shown are the proportion and statistical significance of explained variation in species community composition by pure environmental ([E]) and pure spatial ([S]) variables, together with the joint effect of these two variable sets ([E∩S]). The unexplained proportion of the variation in community composition is expressed as residual variation (R). The significance of the pure environmental and pure spatial components is based on the p-value of F-test for redundancy analysis for the two variable groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.4. Distance decay of community similarity

According to the results of Mantel test, similarity of species composition decreased with an increase in both spatial and environmental distance every month (Fig. 7). Spatial and temporal variation in the similarity decrease was small. Correlation between species and spatial variables was nearly equal and statistically highly significant throughout the study period, whilst the amount and significance of the decrease in similarity along environmental distance increased from May to July. Halving distance of species community composition decreased by 50 m from May's c. 108 m to July's nearly 60 m, nearly half of that in May. Spatial differences in species composition thus increased during the summer. Initial similarity of the species composition was nearly equal in May and June, decreasing slightly toward July. The sharpest and most rapid change in the species composition between separate communities was thus detected in July, whilst the change in community similarity was slowest in May.

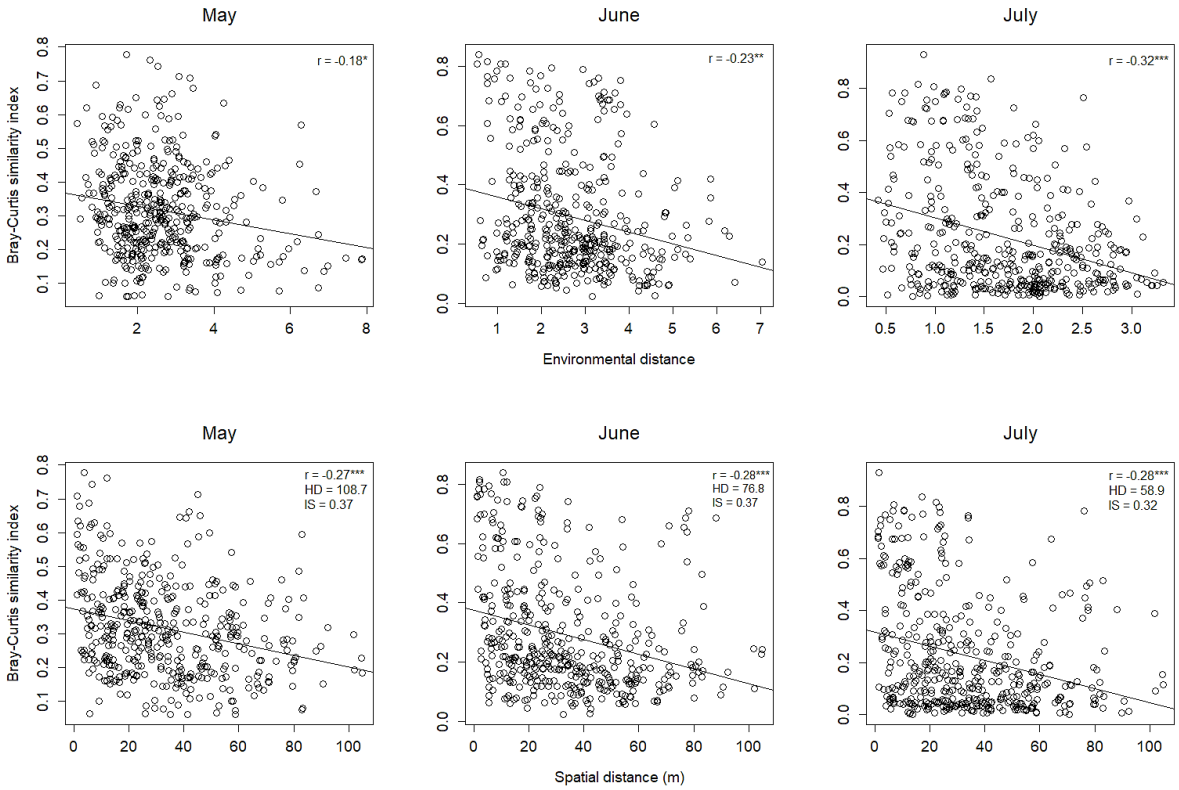


Figure 7. The results of two monthly Mantel tests. Shown is the change in Bray-Curtis similarity index along environmental (above) and spatial (below) distance, together with the correlation coefficient for the relationship between species and both spatial and environmental variables, respectively. For the change in dissimilarity index along spatial distance, the halving distance of community similarity (HD) is also shown. The initial similarity (IS) refers to the dissimilarity index value at a spatial distance of 1 m. The significance of the correlations is based on the p-value: * $p < 0.05$), ** $p < 0.01$, *** $p < 0.001$.

The results of the partial Mantel test differed from the results of the simple Mantel test to some extent (Table 7). This was most likely due to the control for the other variable group simultaneously with correlating the other group of explanatory variables with the response variable, rising the accuracy of the results. In May and June, the similarity of species composition most strongly decreased with an increase in spatial distance, whilst in July the community similarity decreased faster along the environmental distance. The correlation between geographical distance and species composition was strongest in May and weakest in June, whilst the correlation between environment and species composition was weakest in June and strongest in July.

Table 7. The results for monthly Mantel and partial Mantel tests. Shown are the monthly correlation coefficients for pure environmental (env) and pure spatial variables (spat), and the corresponding coefficients for these variable groups when the other variable set was controlled for (env – spat and spat – env, respectively). The statistical significance of the variables is based on the p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Month	Env	Spat	Env - Spat	Spat - Env
May	-0.18*	-0.27***	-0.17*	-0.26***
June	-0.23**	-0.28***	-0.12*	-0.21**
July	-0.32***	-0.28***	-0.29***	-0.24**

8. DISCUSSION

8.1. Rock pool environmental conditions

The studied rock pools were environmentally highly variable throughout the study period. This finding is in line with several previous studies reporting high intra- and inter-pool variations in physiochemical conditions even at a scale of few meters and despite connectivity among pools (Meier & Soininen 2014, Soininen & Meier 2014, Häggqvist & Lindholm 2015). Water nutrient concentrations, temperature and conductivity showed clear and reasonably high spatial variations among the pools especially in May. In June, the variability was less significant, likely reflecting the equalizing effect of rainy and windy weather conditions on water physiochemistry. In May, the early phase of pool hydroperiod following the initial filling probably increased the influence of even small microscale environmental fluctuations in the shallow water with low buffering capacity and volume, resulting in e.g. extremely high nutrient concentrations. Alternatively, random environmental disturbances unequally affecting the pools might have added to the large variability in water physiochemical conditions (Soininen & Meier 2014).

Temporal small-scale variations in local microclimate may have unique effects on local environmental conditions, directly resulting in relative differences in water depth, wave exposure and other physiochemical conditions among the pools of the same disturbance regime (Ganning 1971, Metaxas & Scheibling 1993, Jocque et al. 2010). Hence, pool environmental characteristics were compared with short-term climate data by the Finnish meteorological institute, including daily means of air temperature, rainfall and wind speed at Kumpula meteorological station, Helsinki, covering the period of one week before each sampling (Ilmatieteenlaitos 2017). The climate data clearly reflected temporal fluctuations in pool physiochemical variables. Relatively dry and warm week before the first sampling in May resulted in shallow depth and small area, increasing water temperature, pH and mean nutrient

concentrations and conductivities. In June, heavy rainfalls, high wind speed and relatively low temperature decreased water temperatures and nutrient levels and increased pool depth, with two of the pools merging with each other. In July, air temperatures were the highest, rainfall moderate and wind speed relatively low. Conductivity and nutrient concentrations increased from June while water pH reached its minimum.

8.2. Diatom species richness

Majority of the studied rock pools were characterized by high diatom species richness throughout the study period. Spatial and temporal variations in species richness were high. The highly variable physical and chemical environment of the studied pools likely maintains several ecological niches, supporting multiple species with various environmental adaptations (Soininen & Meier 2014). High spatial and temporal variations in hydroperiod gradient typically increase species diversity by setting demands for highly specialized drought-resistant biota (Therriault & Kolasa 2001, Urban 2004, Williams et al. 2004, O'Neill 2016). The absence of vegetation enables full sunlight to penetrate through the whole water column, rising photosynthetic rates often far beyond those encountered in more shaded habitats and increasing local species richness (Hill 1996, Alahuhta & Aroviita 2016). Alternatively, banks of sedimented propagules in the pools may act as a resisting force for local extinctions often targeted to small aquatic habitats, maintaining viable populations and thus high species richness (Soininen & Meier 2014).

The overall performance of the explanatory variables in the monthly GLM models was rather poor. Diatom species richness is heavily influenced by local environment (Verleyen et al. 2009, Stomp et al. 2011), but the results did not give an indication of high significance of environmental factors on diatoms. The same has previously been observed by other diatom-based studies in lentic habitats (Teittinen & Soininen 2015). Diatom species richness was significantly explained only by water conductivity and pool geographical coordinates. A U-shaped relationship between water conductivity and variation in species richness was observed in June, which deviates from the negative relationship usually observed for these variables (Soininen & Meier 2014, Schröder et al. 2015). This pattern is most likely explained by the interplay between water salinity and pool distance from the sea. Water salinity is an important factor regulating pool biota especially in coastal areas under significant seawater influence (Soininen & Meier 2014), but even minor changes along pool conductivity gradient may have major impact on communities in more inland pools as well (Vanormelingen et al. 2008a).

Majority of the pool diatoms are perhaps either true freshwater species with adaptations to low salinities, or marine species favoring more saline brackish-watered pools. At the ecotone between brackish and freshwater habitats, exotic, salinity-tolerant species are introduced into the less saline pools from the pools close to sea, shifting the dominance from freshwater toward brackish water species along the conductivity gradient (Van Dam et al. 1994, Schröder et al. 2015). The lack of species midway from the coastline toward inland is likely due the low tolerances of these freshwater and marine species against intermediate salinity conditions in these pools. In more sheltered pools farther away from the coast, low conductivities allow persistence of more species not restricted by high salinity, adding to the species diversity of these pools. Conversely, the pools in immediate adjacency to the sea under heavier salinification pressure have likely supported more species with adaptations to marine environment. This pattern is supported by a significant decline in water conductivity with increasing pool distance from the sea in each month, and a significant negative unimodal effect of pool X coordinates in explaining patterns in species richness in July.

High and spatially variable nutrient concentrations were measured throughout the study period but especially in May, largely exceeding concentrations reported form other coastal rock pools in Finland (Häggqvist & Lindholm 2016). Fecal inputs of birds and other visiting animals may temporarily increase especially nitrogen concentrations and multiply the pool nutrient load of shallow coastal pools (Ganning & Wolff 1969, Methratta 2004, Metaxas & Scheibling 1996, Häggqvist & Lindholm 2015). The lack of statistically significant correlations between nutrient concentrations and diatom species richness could be attributed to the lesser importance of nutrients for diatoms in the eutrophicated environment of the northern Baltic Sea with no scarcity of energy supply, as nutrient availability may play a larger role for species diversity in more oligotrophic waters (Soininen & Meier 2014, Virta & Soininen 2017).

8.3. Diatom community composition

The rock pool diatom communities were characterized by highly uneven local abundancies. Majority of the species were extremely rare, sporadically occurring as single cells, while the rest of the community was dominated by few highly abundant species with wide distributions – a general pattern often observed for many diatom communities (Potapova & Charles 2002, Eloranta et al. 2007, Heino et al. 2010, Häggqvist & Lindholm 2015, Smucker & Vis 2011). The dominance of rare species is often a consequence of high endemicity of few species capable of permanently residing in all pools under frequent environmental disturbance (Dethier 1984,

Brendonck et al. 2016). Conversely, species with the highest abundancies tend to be fast-growing habitat generalists or temporary pool inhabitants capable of maintaining high abundancies even under suboptimal environmental conditions and insufficient resources (Therriault & Kolasa 2001, Jocque et al. 2006, Soininen et al. 2007b).

The most abundant species were almost invariably the most common ones, occurring in nearly all pools. Two very common freshwater species (Heino et al. 2010, Soininen & Meier 2014), *Nitzchia microcephala* and *Achnathidium minutissimum*, were among the three most abundant species in each month. This might be at least partly due to the eutrophic state of the pools, since phytoplankton abundance is positively related to total nitrogen and total phosphorus concentrations (Eloranta et al. 2007, Yang et al. 2014, Häggqvist & Lindholm 2015). In May, majority of the species were either singletons (i.e. species occurring as one single cell) or species occurring as multiple cells. In July, none of the species were present in all pools. This might be due to adequate time for highly assembled communities to develop, resulting in increase of the number of highly specialized species during the pool hydroperiod (O'Neill 2016). Alternatively, the scarcity of abundant generalist species could imply the absence of interspecific competition and predation due to frequent environmental disturbances often encountered in small waterbodies (Soininen & Meier 2014).

The influence of the environmental and spatial variables was clearly reflected in diatom species composition. In each monthly RDA, three rock pool categories stood out as the main pool types on their relative environmental and spatial characteristics. Water conductivity was the variable most responsible for variation in diatom communities in the studied pools, explaining by far the largest share of the variance in species composition in each month. A high number of studies support the significance of either water conductivity or salinity for both diatom and coastal rock pool communities (Fránkova et al. 2009, Virtanen & Soininen 2012, Bottin et al. 2014, Meier & Soininen 2014, Liu et al. 2016). Pools closer to the coastline had generally more conductive and relatively less phosphorus-rich water, characterized by species such as *Berkeleya rutilans*, *Cyclotella meneghiniana*, *Diatoma moniliformis*, *Nitzschia fonticola* and *N. frustulum*, species often found either from more saline or less eutrophicated waters (Wojtal & Sobczyk 2012, Virta & Soininen 2017). The diatom community composition was partly affected by total phosphorus, which is in line with previous study results (Soininen 2007, Wu et al. 2014). Warm-watered, phosphorus-rich and less conductive pools located more inland in the study area were occupied by *Achnathidium minutissimum*, *A. subsalsum*, *Gomphonema parvulum* and *Nitzschia*

microcephala, species generally adapted to high phosphorus concentrations (Van Dam et al. 1994, Liu et al. 2016, Mangadze et al. 2016).

A third rock pool group was formed by clearly isolated pools favored by *Nitzschia microcephala* and *N. pusilla*, motile, non-attached small-sized species probably capable of reaching these pools in high abundancies via effective passive dispersal. In fragmented habitats, the total species richness of the separate patches is greater among interconnected than isolated sites (Gilbert et al. 1998, Nekola & White 1999). The degree of pool isolation and the location in respect to pool X coordinates were variably significant for community composition throughout the study, with the emphasize of the latter decreasing toward the end of the summer. This might be due to monthly varying weather conditions. In May and especially in June, stronger winds may have created a transverse gradient reflecting spatial changes in water physiochemical conditions, whilst in July calm and warm weather may have lowered the rate of species wind- and water-aided dispersal, advancing the relative isolation of the pools.

Water pH and temperature were likewise important for community composition, but their influences were more temporally restricted to certain study months. This may be due to the fact that water pH may have a more prominent effect on communities at larger spatial scales such as that of lakes (Pither & Aarssen 2005). Community-scale responses to temperature variation are usually stronger than species-level changes in diversity patterns (Soininen & Weckström 2009). In each month, few pools near the northern edge of the study area stood out either as less conductive or exceptionally diverse in terms of species richness than the other pools. The observed positive relationship of species richness and pool Y coordinates – i.e. the roughly south-north gradient in the study area – in May is probably due to the more exposed location of pools on the southern tip of the inselberg in closer contact to the seawater. A significant share of the studied pools followed a horizontal gradient in water temperature and conductivity along the coastline, the pools with the warmest and least conductive water often located in the more sheltered northern end of the study area, implicating decreasing influence of seawater from south to north.

Temporal changes in the measured environmental and spatial variables were clearly important for the studied rock pool communities, expressed by the significance of the categorical ‘month’ variable. The significance of the spatial and environmental variables for diatom communities were also stronger when considering the whole study period of three months instead of separate months. Besides water physiochemistry and pool location, water depth stood out as a significant

regulator of community composition. Temporal gradient is especially important for communities in pools with varying hydroperiod length (Schneider & Frost 1996). As generally small and shallow pools, monthly temporal fluctuations in water level may have been too weak to be detected, but when combined as a single dataset the influence of pool depth appeared as important.

8.4. Diatom community similarity

Community similarity is usually negatively related to geographical and environmental distances among rock pools and other habitats (Hillebrand et al. 2001, Green et al. 2004, Soininen et al. 2007b, Vanschoenwinkel et al. 2007, Soininen et al. 2011, Astorga et al. 2012, Soininen 2012), albeit contrasting results have also been documented (Urban 2004, Mazaris et al. 2010, Meier & Soininen 2014, Pellowe-Wagstaff & Simonis 2014). Even with seemingly similar environmental and morphological conditions, neighboring rock pools and aquatic habitats in general may significantly differ in their community composition (Dethier 1984, Metaxas & Scheibling 1993, Jenkins & Buikema 1998, Williams et al. 2004, Brendonck et al. 2016). Conversely, similar species assemblages may well inhabit sites that greatly differ in ecological resources (Bradshaw et al. 2002). This was also verified by the significant decay of community similarity along both environmental and geographical gradient from May to July. The increase of significance and strength of decay in community similarity along environmental distance was more pronounced than in geographical distance, reflecting the growing relative importance of environment for species communities from May to July. The studied rock pool communities were more severely altered by variance in geographical than in environmental distance in May, the balance shifting to the opposite in June and July. This is also supported by the results of the partial Mantel test, showing higher significance and correlation of pure spatial variables in May and the opposite in June and July. Nearby communities were hence not similar due to the similarity of their environmental conditions as suggested by some studies, since the significance and importance of pure spatial variables in two of the three months were clearly higher than those of pure environmental ones when controlling for the other variable group.

Several studies report of importance of pool connectedness for species dispersal (Jenkins & Buikema 1998). Interconnected and less isolated pools tend to have more similar species assemblages compared to disconnected or highly isolated pools (Cottenie et al. 2003, Vanschoenwinkel et al. 2007). However, high compositional dissimilarities have been reported for species assemblages among highly interconnected pools and ponds as well (Meier &

Soininen 2014, Häggqvist & Lindholm 2015). Despite assumed high dispersal rates among clustered pools, the dominance of spatial factors in May and the significant decay of community similarity along geographical distance observed in each month indicate that the influence of spatiality clearly occurred between the communities preventing a total homogenization of pool biota (Vanormelingen et al. 2008a, Vanschoenwinkel et al. 2008a). Beta diversity and species turnover rate increased during the summer, as was implied by steadily decreasing monthly halving distances and initial similarities (Soininen 2012). This may stem from the fact that despite effective passive dispersal, benthic diatoms are less capable of dispersing from the water column – especially those species tightly attached to the rocky substratum (Wetzel et al. 2012). Some studies suggest changes in community composition of passive dispersers to occur only beyond 60 m from the nearest pool, independent of the study scale (Vanschoenwinkel et al. 2007). In the current study, the shortest observed halving distance was little less than 60 m, indicating that compositional changes do happen even beneath this distance limit.

Alternatively, the dominance of spatial factors could be attributed to rather calm and dry weather in May, decreasing local water- and wind-aided dispersal rates between the pools. Wind speed and direction affect aerial carrying capacity of passive dispersers especially on coastal areas, while additional rains following pool initial filling enhance water-aided dispersal (Smith 1973, Vanschoenwinkel et al. 2008a, Altermatt et al. 2009, Nabout et al. 2009, Castillo-Escrivà et al. 2017). Although random and sudden overflows may act as a strong dispersal agent allowing species to disperse via water among temporarily connected clustered pools (Jocque et al. 2007, Brendonck et al. 2010), the duration of connections between habitats has sometimes been stated to alter the significance of isolation to pool communities, as very short-lived overflows due to sudden rains are often too temporary for significant dispersal to take place (Vanschoenwinkel et al. 2007). As the experienced overflow events seemed to be rare, of short duration and occurring only between neighboring pools, dispersal among the studied pools most likely occurred via wind, occasionally accompanied with animal-aided dispersal. In small-scale rock pool metacommunities, wind has proven to be the most important dispersal agent, decreasing in carrying capacity only beyond 10 m from the source community (Vanschoenwinkel et al. 2008a, 2008b) – a distance which majority of the studied pools were located within. Thus, the relatively low wind speed and precipitation may have restricted passive dispersal, resulting in higher spatial control in May.

However, as the temporal dominance of environmental gradients imply, dispersal limitation was not a major factor contributing to the species composition of the studied communities. The

significant proportion of pure spatial variables in explaining pool communities at very small spatial scales likely is a result of unmeasured environmental factors increasing the importance of spatiality (Soininen 2015, Liu et al. 2016). The variations in the among-pool distances may have been small enough to allow efficient passive dispersal to take place despite the apparent isolation of the pools (Teittinen & Soininen 2015). According to some studies, the ephemerality of pool connections for the maintenance of community dissimilarity is rather meaningless if very effective dispersal was to take place; significant similarities arise whenever dispersal is allowed by interconnections (Pellowe-Wagstaff & Simonis 2014). Since the otherwise disconnected pools were observed to overflow and even merge with each other after continuous rains in June, this could indeed be the case with the studied diatom communities, resulting in lesser importance of pool isolation to species richness and community composition (Nabout et al. 2009). The increasing beta diversity might also be due to increase in environmental disturbance during the summer. High disturbance restricts competitive exclusions and the presence of generalist species, resulting in the presence of specialists and highly varying species composition between pool communities (Vanschoenwinkel et al. 2010, 2013, Castillo-Escrivà et al. 2017).

8.5. Rock pool metacommunity organization

Although typically mostly under environmental control, freshwater diatom assemblages have been shown to be strongly spatially restricted over varying environmental conditions, at multiple temporal and spatial scales, in lentic and lotic waters and along a salinity gradient from freshwater to marine habitats (Green et al. 2004, Soininen et al. 2004, Soininen 2007, Vyverman et al. 2007, Heino et al. 2010, Bottin et al. 2014, Virta & Soininen 2017). Several findings underline the simultaneous environmental and spatial control over diatom communities, resulting in spatially structured but environmentally variable community (Potapova & Charles 2002, Soininen & Weckström 2009, Verleyen et al. 2009, Bottin et al. 2016, Liu et al. 2016). In the current study, both environmental and spatial variables accounted for significant proportion of the explained variation in community composition each month. The variation in pool diatom communities explained by both environmental and spatial factors was constantly higher than that explained by these factors separately throughout the study period – a pattern previously documented for diatom communities in coastal Baltic Sea region (Virta & Soininen 2017). The effects of pure environmental and pure spatial components were also significant even when the other variable group was controlled for. This result highlights the fact that environmental factors alone are inadequate predictors of patterns in diatom community

composition (Virta & Soininen 2017). In May, pure spatial factors explained slightly more of the variation than pure environmental factors, whilst in June and July the influence of pure environmental factors was higher. Spatial location in respect to geographical coordinates has been detected as one of the most influential spatial factors determining diatom distribution and microbial algal community composition (Soininen 2007, Soininen et al. 2007b, Liu et al. 2016). As with community similarity, connectivity of and distance between suitable habitats, as well as environmental and physical conditions suitable for cell transport all have their share in the success of species dispersal to new regions (Kristiansen 1996, Potapova & Charles 2002).

The dominance of environmental factors in two last study months is in consistence with other studies reporting stronger environmental than spatial control over lentic diatom communities (Beisner et al. 2006, Verleyen et al. 2009, Soininen et al. 2011, Astorga et al. 2012, Bottin et al. 2014, Teittinen & Soininen 2015, Teittinen et al. 2017). The high nutrient concentrations of the studied pools also support the view of partial environmental restriction, since eutrophic habitats tend to be more strongly environmentally structured than oligotrophic ones (Van der Gucht et al. 2007). Overall, the importance of water physiochemical characteristics for the studied communities was highlighted in July. In June and July, additional rains, merging of pools and higher wind speed, along with maturated aquatic invertebrates have likely promoted passive dispersal even at the short timescale of the study, suppressing the relative importance of spatial limitation among the studied diatom communities (Vanschoenwinkel et al. 2008a, Castillo-Escrivà et al. 2017). For ecological generalists the importance of spatial processes for community structure and composition is usually higher than for highly specialized species, which indicates wider species-specific tolerances toward environmental alterations (Potapova & Charles 2002, Vanschoenwinkel et al. 2007, Pandit et al. 2009). This might explain the pattern observed in the higher relative importance of environmental factors in June and July, indicating the late arrival of the most environmentally specialized species toward the end of the hydroperiod.

The significance of both pure environmental and pure spatial components for rock pool diatom communities point toward the dominance of mass effect as the leading metacommunity perspective behind the observed patterns in community composition (Cottenie 2005). This finding is in congruence with other studies suggesting lentic diatom communities as being majorly under the control of both regional dispersal-related and local-scale niche-based processes (Soininen & Weckström 2009, Heino et al. 2010, Bottin et al. 2016). Highly heterogenic environmental conditions likely triggered by random disturbance events serve as a

ground for strong abiotic environmental control, while the minor influence of pool isolation and consequently effective passive dispersal over the relative short distances maintain viable communities even in pools with less favorable ecological conditions for the local diatom flora (Van der Gucht et al. 2007, Vanschoenwinkel et al. 2008b). Species richness in the studied pools likely stays high due to many specialist and endemic species, accompanied with a frequent supply of more generalist species from the surrounding pools preventing communities from homogenization. This results in less similar communities and eventually to higher beta diversity between the pools (Soininen & Heino 2007, Vanschoenwinkel et al. 2013). Despite the lack of connecting waterways between the pools, dispersal is not strongly limited by geographical barriers in this small-scale rock pool metacommunity (Cottenie et al. 2003, Vanschoenwinkel et al. 2007). Frequent and efficient dispersal likely accompanied with some unmeasured environmental variables lowers the relative importance of environmental factors while maintaining a significant effect of spatiality, resulting in environmentally restricted metacommunity with weaker effect of spatiality.

8.6. Reliability of the results

Despite standardized sampling methods, some inaccuracies may have inevitably occurred during sampling. Especially some of the smallest-sized cells may have been misidentified, and some of the cells were impossible to identify due to the lateral position of the cell. Identification of diatoms is often complexed by the low proportion of large-celled species in comparison to smaller ones. Larger-sized species are rarely encountered and often underrepresented in microbial samples, while small and abundant species are prone to be overrepresented (Lowe & Pan 1996, Snoeijs et al. 2002). In addition, due to the high cell abundance typical of diatoms, species identification based on a fixed number of counted cells never truly reflects the real species richness in the given study site. Rather, as it is practically impossible to prove that a species is absent from a site, the count only serves as an approximation of the species evenness in the community (Stevenson 1996, Martiny et al. 2006). In addition, the presence of some of the species may be sporadic, decreasing the probability for detecting that species (Bottin et al. 2014).

The quantification of total phosphorus and total nitrogen concentrations in June was done by different person than in May and July, theoretically adding some divergences to the results. However, as the work followed the exact same, highly standardized methods and was supervised by the same laboratory personnel as in the two other months, the noise probably

added to the results is likely of minor importance. Of the morphological variables, pool area was an approximation estimated from the measured maximum pool length and width, likely adding some level of inaccuracy to the study results. Likewise, the geographical coordinates and the degree of pool isolation were estimated from the grid map of the study area, which likely deviated from the natural distances to some extent. However, all these measures gave an approximation of the relative distances between the studied pools, as well as the relative size distribution.

The lack of significant relationships between diatom species richness and the environmental variables, together with the high residual variation in community composition could be explained by exclusion of some key environmental factors not included in the explanatory variables. This might be true in the current study as well since a few important variables were obviously left unmeasured. This might lower especially variation explained by pure environmental factors among the studied communities (Soininen 2015, Liu et al. 2016). For example, dissolved oxygen concentration or biotic interactions were not quantified. In eutrophic waters the importance of environmental resources in regulating community composition is usually larger than that of biotic interactions (Droop 1953, Soininen & Meier 2014). The high species richness and environmental heterogeneity among the studied rock pools suggest that the negative effects of grazing and interspecific competition are largely overcame by this high resource availability, allowing several species to coexist at the same site without notable competition even in the case of partly overlapping ecological niches (McGormick 1996). Additionally, all other important chemical variables previously found as primary regulators of diatoms were included in the environmental variable set (Heino et al. 2010). It thus seems unlikely that the lack of significant relationships in the data would have been due to inadequate measures of pool physiochemistry. Moreover, inclusion of spatial variables in the study not only suppresses the relative importance of environmental factors to more reliable level, but also increases the proportion of total explained variation among the communities (Liu et al. 2016).

Although rock pools usually well compare to other larger and more complex aquatic systems, some caution should be taken when directly upscaling ecological results from these habitats. Along with scale-dependence, the relative importance of environmental and spatial control is partly habitat-dependent (Cottenie 2005, Soininen 2007). As a highly disturbed environment, majority of pool biota consists of highly specialized species – a situation which rarely is encountered in other aquatic habitats (Vanschoenwinkel et al. 2013). Additionally, the degree of habitat isolation greatly differs between lotic and lentic waters. Lotic waters are often better

interconnected, and distribution of species is less affected by spatiality. In lentic waters, low connectivity between habitat patches limits species dispersal, resulting in stronger spatial control over diatom communities (Soininen 2007, Soininen & Weckström 2009, Soininen 2012, Bottin et al. 2014). The relative importance of environmental and spatial factors also depends on the dispersal mode. Contrary to actively dispersing species, passive dispersers are more spatially restricted due their lesser capacity of introducing themselves to new habitats, being more affected by spatial patterns of rock pool metacommunity (Beisner et al. 2006, Vanschoenwinkel et al. 2007, Castillo-Escrivà et al. 2017). Within passive dispersers, the relative dispersal ability and body size of organisms further determines the rate of dispersal, with microbial phytoplankton typically having the highest dispersal rates and thus being least restricted by spatial factors (Soininen et al. 2011, Padial et al. 2014).

9. CONCLUSIONS

The studied rock pools were morphologically similar but environmentally heterogeneous, with high spatial and temporal variation in water physiochemical characteristics. The geographical distance from the nearby Baltic Sea formed a spatial gradient affecting water conductivity and species richness among the pools. The overall species richness was high. Majority of the detected diatom species were rare, with few species dominating the rock pool communities throughout the study period. In June, conductivity was the sole driver of species richness, whilst in May and July the variation in diversity patterns mostly resulted from differences in pool geographical location (Fig. 1). Through the study period, diatom community composition was likewise mostly affected by water conductivity and the spatial location of the pools along the coast. Water physiochemistry increased in importance during the summer, while temporal and spatial gradients were especially responsible for the overall pattern in community composition during the whole study period.

The diatom species composition and community similarity among the studied rock pools were significantly affected by both environment and space every month, with environment overcoming the effect of spatiality after the first sampling event in May. The similarity in species composition decreased both along environmental and spatial distance, the latter being stronger in two of the three study months. Along with high species richness, the diatom communities were characterized by steadily increasing beta diversity toward the end of the summer. The results of this study support the dominance of mass effect as the leading metacommunity perspective behind the assembly of small-scale rock pool communities, with

both environmental heterogeneity and efficient dispersal responsible for structuring of local diatom pool communities. The highly variable and disturbed physiochemical environment likely contributed to the high local species richness, while the efficient dispersal over the relatively short distances between the pools assisted with suitable weather conditions for passive dispersal offered an additional supply of species from neighboring pools. Despite the obviously effective dispersal, increasing distance decay of community similarity along geographical distance during the summer possibly indicate the influence of some sort of restricted dispersal among the communities, preventing total homogenization of the diatom flora between the studied pools. However, most of the observed spatial control over the studied communities is likely a cause of unmeasured environmental variables, increasing the significance and relative importance of spatial factors.

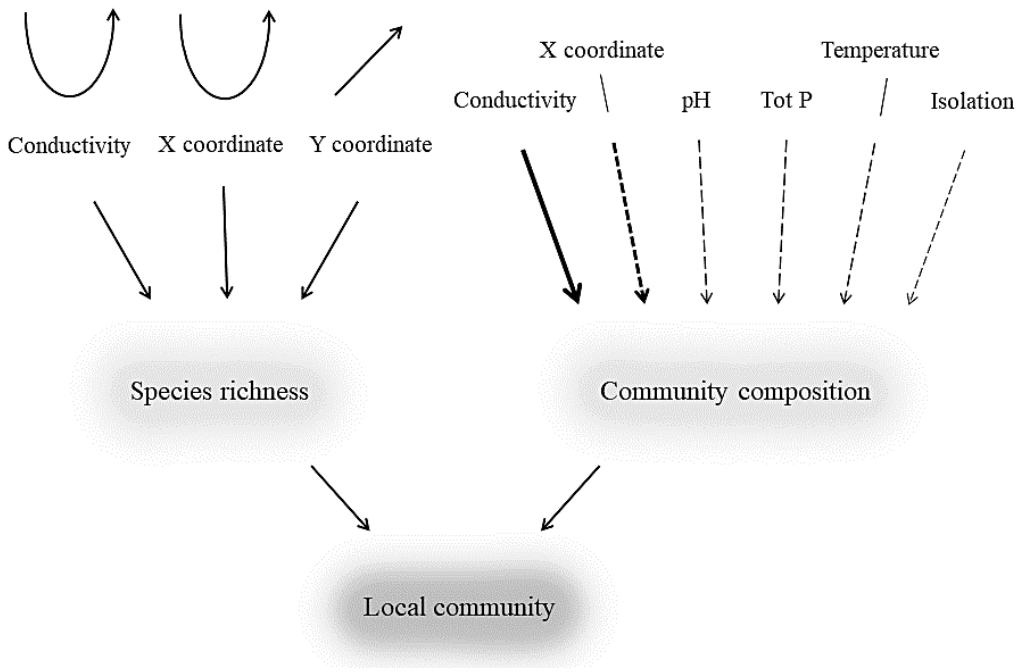


Figure 1. The explanatory variables most responsible for variation in species richness and community composition among the studied rock pool diatom communities. Water conductivity and pool X coordinates has a U-shaped and pool Y coordinates a positive linear relationship with species richness, respectively. For community composition, the gradually decreasing arrow width is proportional to the statistical significance of the explanatory variable, ranging from more significant for conductivity ($p < 0.001$) and X coordinates ($p < 0.01$) to weakly significant ($p < 0.05$) for the rest of the variables. Solid arrow indicates that variable was of importance for community composition in each month, while dashed arrows indicate that the variable was important only in two (X coordinates) or one month (the rest of the explanatory variables).

10. ACKNOWLEDGEMENTS

Thanks to my supervisors Janne Soininen and Anette Teittinen for adept guidance, advice and assistance throughout the study. Thanks to Hanna Reijola and other personnel of the department laboratory for assistance with the treatment of the nutrient samples. Thanks to Simon Strömgård for determination of the nutrient concentrations of the June's water samples. Many thanks to my family for support. Last but not least, thanks to all other anonymous persons for unlimited support and advice throughout the working process – you know who you are.

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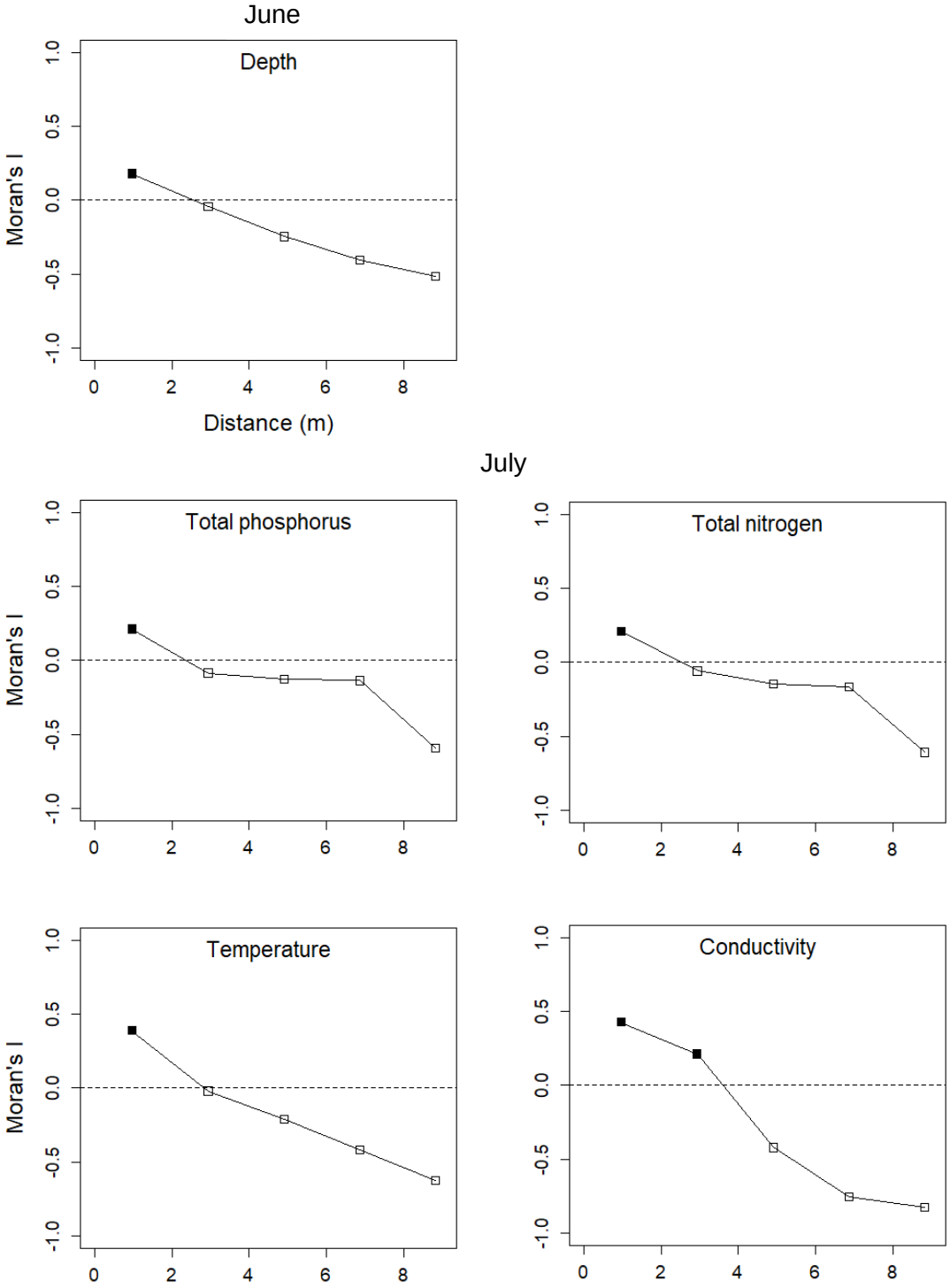
APPENDICES

APPENDIX 1. The Spearman rank correlation coefficients and the statistical significance of correlations for the response variable and environmental and spatial variables in June (upper) and July (lower). Shown are three significance levels according to the p-value of each pairwise correlation: - non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Bolded correlation coefficients are statistically significant ($p < 0.05$).

	Total P	Total N	pH	Conductivity	Temperature	Lenght	Width	Depth	Area	Volume	X coordinate	Y coordinate	Isolation	Species richness
Total P		**0.57**	0.12	-0.04	0.24	-0.06	-0.05	0.09	-0.06	0.02	0.19	-0.09	0.11	-0.06
Total N	**		-0.06	**-0.48**	**0.42**	-0.24	-0.22	-0.10	-0.25	-0.19	0.18	**0.47**	0.13	0.09
pH	-	-		**0.44**	-0.24	-0.23	-0.33	-0.23	-0.26	-0.30	0.01	-0.29	-0.01	-0.16
Conductivity	-	**	*		**-0.63**	0.06	0.07	-0.14	0.09	0.04	**-0.43**	**-0.79**	-0.13	-0.08
Temperature	-	*	-	***		0.01	-0.22	-0.06	-0.17	-0.12	0.09	**0.61**	0.05	0.01
Lenght	-	-	-	-	-		**0.65**	**0.51**	**0.90**	**0.90**	0.00	-0.01	-0.05	0.26
Width	-	-	-	-	-	***		**0.55**	**0.90**	**0.86**	0.20	-0.28	-0.27	-0.06
Depth	-	-	-	-	-	**	**		**0.61**	**0.72**	**0.45**	-0.08	**-0.39**	-0.09
Area	-	-	-	-	-	***	***	***		**0.98**	0.14	-0.17	-0.22	0.11
Volume	-	-	-	-	-	***	***	***	***		0.19	-0.14	-0.26	0.09
X coord.	-	-	-	*	-	-	-	*	-	-		0.04	-0.23	-0.17
Y coord.	-	**	-	***	***	-	-	-	-	-	-		0.26	0.18
Isolation	-	-	-	-	-	-	-	*	-	-	-	-		0.19
Sp. richness	-	-	-	-	-	-	-	-	-	-	-	-	-	

	Total P	Total N	pH	Conductivity	Temperature	Length	Width	Depth	Area	Volume	X coordinate	Y coordinate	Isolation	Species richness
Total P		**0.74**	0.11	-0.28	**0.64**	-0.25	-0.06	-0.11	-0.19	-0.21	0.36	-0.06	-0.18	-0.17
Total N	***		-0.12	-0.32	**0.79**	-0.28	-0.18	-0.31	-0.26	-0.29	0.33	0.12	-0.32	-0.03
pH	-	-		0.13	-0.23	**-0.44**	-0.23	-0.19	-0.33	-0.31	-0.20	0.09	0.28	-0.01
Cond.	-	-	-		-0.36	0.05	-0.10	-0.16	-0.01	-0.07	**-0.65**	**-0.53**	0.01	-0.10
Temp.	***	***	-	-		-0.31	**-0.37**	-0.27	**-0.37**	**-0.38**	**0.39**	0.14	-0.17	0.01
Lenght	-	-	*	-	-		**0.77**	**0.59**	**0.92**	**0.91**	0.01	-0.04	-0.08	**-0.41**
Width	-	-	-	-	*	***		**0.64**	**0.94**	**0.91**	0.13	-0.12	-0.29	**-0.40**
Depth	-	-	-	-	-	***	***		**0.68**	**0.77**	0.31	0.00	-0.25	-0.22
Area	-	-	-	-	*	***	***	***		**0.98**	0.11	-0.12	-0.23	**-0.43**
Volume	-	-	-	-	*	***	***	***	***		0.15	-0.05	-0.21	**-0.38**
X coord.	-	-	-	***	*	-	-	-	-	-		0.04	-0.23	-0.10
Y coord.	-	-	-	**	-	-	-	-	-	-	-		0.26	0.14
Isolation	-	-	-	-	-	-	-	-	-	-	-	-		0.27
Sp. richness	-	-	-	-	-	*	*	-	*	*	-	-	-	

APPENDIX 2. Statistically significant spatial correlograms for environmental variables in June and July. Shown are the Moran's *I* correlation coefficients [-1, 1] for observation values in each of the five distance classes separated by 2.5 m. Statistically significant correlations (Bonferroni-corrected p-value < 0.05) in each distance class are marked as a black square.



APPENDIX 3. Values of the environmental variables for the studied rock pools in each month. Pool area and volume are calculated from the measures of maximum pool length, width and depth.

May										
Pool	Tot P (µg/l)	Tot N (µg/l)	pH	Conduct. (µS/cm)	Temp. (°C)	Length (m)	Width (m)	Depth (m)	Area (m^2)	Volume (m^3)
1	107.9	762.5	9.1	5360	19.0	0.19	0.35	0.09	0.26	0.02
2	484.6	3087.5	9.9	10075	19.6	0.20	0.35	0.13	0.21	0.03
3	273.2	2562.5	9.5	3095	20.1	0.20	0.50	0.17	2.18	0.37
4	1208.1	2125.0	9.3	6166	19.7	0.20	0.55	0.12	0.99	0.12
5	262.3	7800.0	9.3	16741	22.8	0.23	1.00	0.15	0.90	0.14
6	319.3	5900.0	8.6	14460	18.6	0.19	0.75	0.08	0.94	0.08
7	131.2	3500.0	9.5	7260	21.8	0.22	0.50	0.17	1.40	0.24
8	267.3	2637.5	8.7	3758	18.5	0.19	0.45	0.23	0.56	0.13
9	182.3	2025.0	9.0	2304	19.5	0.20	0.60	0.28	0.39	0.11
10	132.0	2425.0	9.5	5970	20.9	0.21	0.85	0.30	1.21	0.36
11	437.1	4750.0	9.1	3380	20.7	0.21	0.85	0.23	1.02	0.23
12	123.1	1787.5	9.5	1723	22.0	0.22	2.40	0.29	6.72	1.95
13	49.1	737.5	9.3	8439	21.7	0.22	1.40	0.20	2.38	0.48
14	164.0	2037.5	9.8	1134	21.0	0.21	4.80	0.34	33.12	11.26
15	135.1	2650.0	9.4	1485	22.5	0.23	0.50	0.20	0.83	0.17
16	848.9	8300.0	9.5	3144	24.7	0.25	0.55	0.10	0.33	0.03
17	378.7	4250.0	8.2	458	22.3	0.22	0.20	0.14	0.09	0.01
18	384.6	4150.0	9.4	219	22.0	0.22	0.15	0.15	0.15	0.02
19	507.1	2175.0	9.6	9054	22.9	0.23	0.80	0.13	1.16	0.15
20	71.0	1437.5	8.6	744	20.4	0.20	2.40	0.29	13.20	3.83
21	451.7	7650.0	8.6	134	19.5	0.20	0.30	0.05	0.15	0.01
22	1448.3	3891.7	8.6	188	26.2	0.26	0.35	0.05	0.42	0.02
23	187.4	2012.5	8.1	400	21.3	0.21	1.00	0.25	10.70	2.68
25	689.0	5550.0	9.5	472	21.3	0.21	0.30	0.15	0.17	0.02
26	66.3	812.5	8.6	8745	22.0	0.22	0.35	0.17	0.35	0.06
27	54.1	1425.0	8.6	1275	22.4	0.22	0.20	0.15	0.15	0.02
28	225.2	2112.5	9.8	152	19.7	0.20	0.30	0.17	0.21	0.04
29	448.0	3950.0	9.7	752	22.2	0.22	0.25	0.14	0.18	0.02
30	1016.8	8750.0	9.1	348	20.3	0.20	0.15	0.08	0.12	0.01
35	298.5	3600.0	9.5	1498	22.7	0.23	0.45	0.16	0.68	0.11

June										
Pool	Tot P (µg/L)	Tot N (µg/L)	pH	Conduct. (µS/cm)	Temp. (°C)	Length (m)	Width (m)	Depth (m)	Area (m^2)	Volume (m^3)
1	24.1	656.3	8.9	8872	14.8	0.85	0.55	0.11	0.47	0.05
2	184.6	2050.0	9.0	10754	17.1	0.68	0.37	0.14	0.25	0.04
3	87.4	1150.0	9.0	8004	17.1	4.80	0.75	0.30	3.60	1.08
4	72.8	950.0	9.6	10140	17.7	1.83	0.66	0.13	1.21	0.16
5	105.6	1550.0	9.3	9890	17.6	3.90	1.50	0.21	5.85	1.23
6	41.4	1100.0	9.5	8912	17.2	1.45	1.06	0.14	1.54	0.22
7	17.3	750.0	10.0	8960	17.7	2.95	0.59	0.25	1.74	0.44
8	33.4	850.0	9.5	7932	17.8	1.20	0.45	0.26	0.54	0.14
9	66.4	900.0	8.8	5202	17.3	0.65	0.63	0.30	0.41	0.12
10	363.1	3900.0	9.4	8347	19.4	1.78	1.03	0.40	1.82	0.73
11	187.0	2050.0	8.5	5520	18.6	1.20	0.86	0.23	1.03	0.24
12	78.4	1100.0	8.5	3823	18.8	3.55	1.62	0.34	5.73	1.95
13	19.1	500.0	8.8	8732	16.4	2.00	1.60	0.24	3.20	0.77
14	42.4	1083.3	7.4	1277	19.2	6.80	5.40	0.36	36.72	13.22
15	213.5	1400.0	7.6	3203	20.4	1.80	0.50	0.24	0.90	0.22
16	60.6	1350.0	8.7	3913	20.6	0.70	0.65	0.15	0.46	0.07
17	59.2	5175.0	8.9	533	19.4	1.85	0.70	0.21	1.30	0.27
18	31.6	1233.3	8.1	1278	20.0	0.97	0.30	0.20	0.29	0.06
19	94.4	1200.0	9.3	7409	20.6	2.10	0.50	0.15	1.05	0.16
20	72.5	1393.8	8.0	115	21.3	5.50	3.20	0.37	17.60	6.51
21	67.3	6350.0	7.7	126	19.6	0.65	0.52	0.13	0.34	0.04
22	36.4	1000.0	7.2	63	20.5	3.60	1.35	0.20	4.86	0.97
23	54.3	1731.3	7.2	410	19.0	16.20	1.50	0.30	24.30	7.29
25	60.7	3625.0	8.7	207	16.4	1.60	1.30	0.27	2.08	0.56
26	27.0	983.3	8.6	8475	18.2	2.00	0.43	0.18	0.86	0.15
27	50.6	1512.5	8.8	178	18.2	1.05	0.50	0.22	0.53	0.12
28	99.3	1956.3	9.8	62	19.5	0.90	0.42	0.22	0.38	0.08
29	64.2	2825.0	9.0	384	20.8	1.00	0.30	0.19	0.30	0.06
30	80.7	7425.0	9.8	119	20.7	0.97	0.20	0.12	0.19	0.02
35	68.7	2150.0	7.6	307	19.2	1.70	0.48	0.19	0.82	0.16

July										
Pool	Tot P (µg/L)	Tot N (µg/L)	pH	Conduct. (µS/cm)	Temp. (°C)	Lenght (m)	Width (m)	Depth (m)	Area (m^2)	Volume (m^3)
1	50.1	550.0	8.0	8075	17.4	0.82	0.40	0.09	0.33	0.03
2	101.4	1100.0	8.3	12203	18.0	0.71	0.35	0.14	0.25	0.03
3	127.5	1200.0	7.9	9430	18.8	4.65	0.75	0.30	3.49	1.05
4	444.5	3300.0	7.9	14383	20.0	1.80	0.55	0.09	0.99	0.09
5	174.9	2500.0	8.1	4509	19.0	3.90	1.82	0.19	7.10	1.35
6	152.8	1800.0	7.9	1429	19.5	1.42	1.02	0.11	1.45	0.16
7	58.9	1600.0	7.7	2404	19.6	2.90	0.62	0.22	1.80	0.40
8	131.7	1750.0	7.6	1564	19.9	1.17	0.47	0.26	0.55	0.14
9	200.1	2150.0	7.9	151	19.8	0.66	0.60	0.29	0.40	0.11
10	265.7	3450.0	8.3	847	19.9	1.73	1.05	0.32	1.81	0.58
11	268.8	2600.0	7.7	532	19.9	1.20	0.85	0.24	1.02	0.24
12	80.7	1443.8	7.6	371	19.6	6.70	3.05	0.29	20.44	5.93
13	22.2	400.0	8.0	8920	17.1	1.70	1.35	0.22	2.30	0.50
14	204.9	1500.0	7.8	229	19.1	6.60	5.30	0.34	34.98	11.89
15	96.1	1650.0	7.3	10390	19.9	1.80	0.52	0.22	0.94	0.21
16	250.8	2450.0	8.5	12474	19.8	0.64	0.58	0.11	0.37	0.04
17	724.8	6100.0	7.8	188	19.9	1.85	0.85	0.22	1.57	0.35
18	104.0	3400.0	6.9	1039	20.5	1.02	0.34	0.16	0.35	0.06
19	39.3	1687.5	7.5	8972	16.9	2.30	0.68	0.20	1.56	0.31
20	111.2	1350.0	7.6	109	19.4	5.60	3.60	0.36	20.16	7.26
21	583.6	5050.0	7.8	39	20.5	0.60	0.37	0.09	0.22	0.02
22	55.3	2616.7	6.7	48	19.9	3.55	1.23	0.18	4.37	0.79
23	150.9	1450.0	7.1	90	18.9	15.90	1.85	0.29	29.42	8.53
25	147.7	1700.0	8.4	168	18.4	1.55	1.15	0.26	1.78	0.46
26	28.1	1333.3	8.5	8965	15.8	2.20	0.80	0.27	1.76	0.48
27	75.0	1600.0	8.0	127	19.3	1.05	0.50	0.21	0.53	0.11
28	162.9	1800.0	9.5	38	19.8	0.80	0.41	0.19	0.33	0.06
29	168.2	1700.0	8.9	101	20.6	0.95	0.31	0.18	0.29	0.05
30	413.6	6100.0	7.9	151	20.7	0.85	0.16	0.11	0.14	0.01
35	48.4	700.0	8.0	9100	18.3	1.65	0.50	0.20	0.83	0.17

APPENDIX 4. Values of the spatial variables for the studied rock pools. Spatial coordinates and isolation are estimated distances from the grid map of the study area. Pool isolation is calculated as the mean distance to 5 closest pools.

Spatial data			
Pool	X coordinate (m)	Y coordinate (m)	Isolation (m)
1	1.6	1.8	16.3
2	5.0	1.4	15.9
3	10.0	13.8	10.9
4	5.0	15.6	9.8
5	5.8	26.0	3.5
6	7.2	26.0	2.8
7	7.4	27.2	2.7
8	9.4	26.6	3.0
9	9.0	25.6	3.2
10	8.0	32.6	5.4
11	9.4	35.4	6.6
12	8.0	38.8	7.1
13	2.2	43.6	8.3
14	7.0	46.6	6.2
15	4.6	49.4	4.1
16	3.4	49.8	4.4
17	6.2	53.0	4.6
18	5.6	54.2	4.8
19	3.2	59.2	7.5
20	9.0	62.8	7.1
21	10.4	64.8	7.4
22	10.0	68.0	7.6
23	6.8	72.4	7.6
25	8.0	81.0	5.9
26	2.2	84.8	8.5
27	8.0	84.6	6.9
28	11.4	83.0	7.8
29	6.4	103.6	16.9
30	6.6	106.2	18.9
35	2.4	77.4	8.1